

Docket No. 1878-4051

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

UTILITY APPLICATION AND APPLICATION FEE TRANSMITTAL (1.53(b))

ASSISTANT COMMISSIONER FOR PATENTS Box Patent Application Washington, D.C. 20231

Sir:

Transmitted herewith for filing is the patent application of

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For:

INSULIN AND IGF-1 RECEPTOR AGONISTS AND ANTAGONISTS

Enclosed are:

[X] 145 page(s) of specification, 1 page(s) of Abstract, 44 page(s) of claims

[X] 199 sheets of drawing

[] formal [X] informal

[X] 8 page(s) of Declaration and Power of Attorney

[X] Unsigned

	[] Nev [] Coj	wly Executed py from prior application					
		[] Deletion of inventors including Signed Statement under 37 C.F.R. § 1.63(d)(2)					
[X]	Incorporation by Reference: The entire disclosure of the prior application is considered as being part of the disclosure of the accompanying application and is incorporated herein by reference.						
[]	Microfiche Computer Program (Appendix)						
[]	page(s) of Sequence Listing						
	[]	computer readable disk containing Sequence Listing Statement under 37 C.F.R. § 1.821(f) that computer and paper copies of the Sequence Listing are the same					
[]	Claim for Priority						
[]	Certified copy of Priority Document(s)						
	[]	English translation documents					
[]	Inform	Information Disclosure Statement					
	[]	Copy of cited references					
	[]	Copy of PTO-1449 filed in parent application serial No					
[]	Prelim	Preliminary Amendment					
[X]	Return receipt postcard (MPEP 503)						
[]	Assignment Papers (assignment cover sheet and assignment documents)						
	[]	A check in the amount of \$40.00 for recording the Assignment.					
	[]	Assignment papers filed in parent application Serial No.					
	[]	Certification of chain of title pursuant to 37 C.F.R. § 3.73(b).					
[X]	This is a [] continuation [] divisional [X] continuation-in-part (C-I-P) of prior application serial no. 09/146,127.						
	[]	Cancel in this application original claims of the parent application before calculating the filing fee. (At least one original independent claim must be retained for filing purposes.)					
	[]	A preliminary Amendment is enclosed. (Claims added by this Amendment have been properly numbered consecutively beginning with the number following the highest numbered original claim in the prior application.					
[X]	The status of the parent application is as follows:						
	A Petition For Extension of Time and a Fee therefor has been or is being filed in the parent application to extend the term for action in the parent application until						
	[]	A copy of the Petition for Extension of Time in the co-pending parent application is attached.					

	[X] No Petition For Extension of Time and Fee therefor are necessary in the co-pending parent application.								
[]	Please abandon the parent application at a time while the parent application is pending or at a time when the petition for extension of time in that application is granted and while this application is pending has been granted a filing date, so as to make this application co-pending.								
	[] Transfer the drawing(s) from the patent application to this application.								
[]	Amend the specification by inserting before the first line the sentence: This is a [] continuation [] divisional [] continuation-in-part of co-pending application Serial No.								
	I.	CALCULATION OF APPLIC	ATION FEE	(For Other Than A Sma	all Entity)	Basic Fee			
		Number Filed		Number Extra	Rate	\$690.00			
Total Claims		268	-20=	248	x\$18.00	\$4464.00			
Indeper	ndent	50	- 3=	47	x\$78.00	\$3666,00			
		dent Claims							
-	-	[X] yes [] no		Additional Fee = Add'l Fee =	\$260.00 NONE	\$260.00			
					Total	: <u>\$9080.00</u>			
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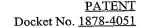
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THE STATE SECTION



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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):

Arthur J. Blume, et al.

Group Art Unit: TBA

Serial No.:

TBA

Examiner: TBA

Filed:

March 29, 2000 (Herewith)

For:

INSULIN AND IGF-1 RECEPTOR AGONISTS AND ANTAGONISTS

EXPRESS MAIL CERTIFICATE (37 C.F.R. §1.10)

Express Mail Label No.: EJ607481229US

Date of Deposit: March 29, 2000

I hereby certify that the following attached paper(s) and/or fee for:

Utility Application and Application Fee Transmittal (§ 1.53(b)); (3 Pages (In Duplicate)); 1.

Specification (145 Pages); Abstract (1 Page); Claims (44 Pages); 2.

Informal Drawings (Figures 1-74); (199 Pages); 3.

Combined Declaration and Power of Attorney (8 Pages); 4.

Check (\$9080.00); and 5.

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is/are being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. §1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

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INSULIN AND IGF-1 RECEPTOR AGONISTS AND ANTAGONISTS

This application is a continuation-in-part of U.S. Application Serial No. 09/146,127, filed September 2, 1998, which is incorporated by reference in its entirety.

5 I. FIELD OF THE INVENTION

This invention relates to the field of hormone receptor activation or inhibition. More specifically, this invention relates to the identification of molecular structures, especially peptides, which are capable of acting at either the insulin or insulin-like growth factor receptors as agonists or antagonists. Also related to this invention is the field of molecular modeling whereby useful molecular structures are derived from known structures.

II. BACKGROUND OF THE INVENTION

Insulin is a potent metabolic and growth promoting hormone that acts on cells to stimulate glucose, protein, and lipid metabolism, as well as RNA and DNA synthesis. A well-known effect of insulin is the regulation of the level of glucose at a whole body level. This effect by insulin occurs predominantly in liver, fat, and muscle. In liver, insulin stimulates glucose incorporation into glycogen and inhibits the production of glucose. In muscle and fat, insulin stimulates glucose uptake, storage, and metabolism. Disruptions of glucose utilization are very common in the population in giving rise to diabetes.

Signal transduction in target cells is initiated by binding of insulin to a specific cell-surface receptor, the insulin receptor (IR). The binding leads to conformational changes in the extracellular domain of the receptor, which are transmitted across the cell membrane and result in activation of the receptor's tyrosine kinase activity. This, in turn, leads to autophosphorylation of the insulin receptor's tyrosine kinase, and the binding of soluble effector molecules that contain SH2 domains such as

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phophoinositol-3-kinase, Ras GTPase-activating protein, and phospholipase C_{γ} to IR (Lee and Pilch, 1994).

Insulin-like growth factor 1 (IGF-1) is a small, single-chain protein (MW = 7,500 Da) that is involved in many aspects of tissue growth and repair, and recently has been implicated in various forms cancer including prostrate, breast, colorectal, and ovarian. It is similar in size, sequence and structure to insulin, but has 100-1,000-fold lower affinity for the insulin receptor (Mynarcik *et al.*, 1997).

Clinically, recombinant human IGF-1 has been investigated for the treatment of several diseases, including type I diabetes (Carroll *et al.*, 1997; Crowne *et al.*, 1998), amyotropic lateral sclerosis (Lai *et al.*, 1997), and diabetic motor neuropathy (Apfel and Kessler, 1996). Other potential therapeutic applications of IGF-1 such as osteoporosis (Canalis, 1997), immune modulation (Clark, 1997) and nephrotic syndrome (Feld and Hirshberg, 1996) are being examined.

A number of studies have analyzed the role of natural IGF-1 in various disease states. Most interestingly, several reports have shown that IGF-1 promotes the growth of normal and cancerous prostate cells both *in vitro* and *in vivo* (Angelloz-Nicoud and Binoux, 1995; Figueroa *et al.*, 1995; Torring *et al.*, 1997). Additionally, elevated serum IGF-1 levels have been connected with increased risks of prostate cancer, and may be an earlier predictor of cancer than is prostate-specific antigen (PSA) (Chan *et al.*, 1998). Recent studies have indicated a connection between IGF-1 and other cancers such as breast, colorectal, and ovarian. Serum IGF-1 levels are regulated by the presence of IGF binding proteins (IGFBP) which bind to IGF-1 and prevent its interaction with the IGF-1R (reviewed in Conover, 1996 and Rajaram *et al.*, 1997). Interestingly, PSA has been shown to be a protease that cleaves IGFBP-3, resulting in an increase of free IGF-1 in serum (Cohen *et al.*, 1992; Cohen *et al.*, 1994; Lilja, 1995). Clearly, regulation of IGF-1R activity can play an important role in several disease

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states, indicating that there are potential clinical applications for both IGF-1 agonists and antagonists.

The type-1 insulin-like growth-factor receptor (IGF-1R) and insulin receptor (IR) are related members of the tyrosine-kinase receptor superfamily of growth factor receptors. Both types of receptors are composed of two α and two β subunits which form a disulfide-linked heterotetramer (β - α - α - β). They have an extracellular ligand binding domain, a single transmembrane domain, and a cytoplasmic domain displaying the tyrosine kinase activity. The extracellular domain is composed of the entire subunits and a portion of the N-terminus of the β subunits, while the intracellular portion of the β subunits contains the tyrosine kinase domain. Besides IR and IGF-1R, the other known member of the IR family is the insulin-related receptor (IRR), for which no natural ligand is known.

While similar in structure, IGF-1 and insulin receptors serve different physiological functions. The IR is primarily involved in metabolic functions whereas the IGF-1R mediates growth and differentiation. However, both insulin and IGF-1 can induce both mitogenic and metabolic effects. Whether each ligand elicits both activities via its own receptor, or whether insulin exerts its mitogenic effects through its weak affinity binding to the IGF-1 receptor, and IGF-1 its metabolic effects through the insulin receptor, remains controversial. (De Meyts, 1994).

The insulin receptor is a glycoprotein having molecular weight of 350-400 kDa (depending of the level of glycosylation). It is synthesized as a single polypeptide chain and proteolytically cleaved yielding the disulfide-linked monomer α - β insulin receptor. Two α - β monomers are linked by disulfide bonds between the α -subunits to form a dimeric form of the receptor (β - α - α - β -type configuration). The α subunit is comprised of 723 amino acids, and it can be divided into two large homologous domains, L1 (amino acids 1-155) and L2 (amino acids 313-468), separated by a cysteine rich region (amino acids 156-312) (Ward *et al.*, 1995). Many determinants

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of insulin binding seem to reside in the α -subunit. A unique feature of the insulin receptor is that it is dimeric in the absence of ligand.

The sequence of IR is highly homologous to the sequence of the type-1 insulin-like growth factor receptor (IGF-1R). The homology level varies from about 40% to 70%, depending on the position within the α -subunit. The three-dimensional structures of both receptors may therefore be similar. The crystal structure of the first three domains of IGF-1R has been determined (Garrett *et al.*, 1998). The L domains consist of a single-stranded right-handed β -helix (a helical arrangement of β -strands), while the cysteine-rich region is composed of eight disulfide-bonded modules.

The β -subunit of the insulin receptor has 620 amino acid residues and three domains: extracellular, transmembrane, and cytosolic. The extracellular domain is linked by disulfide bridges to the α -subunit. The cytosolic domain includes the tyrosine kinase domain, the three-dimensional structure of which has been solved (Hubbard *et al.*, 1994).

To aid in drug discovery efforts, a soluble form of a membrane-bound receptor was constructed by replacing the transmembrane domain and the intracellular domain of IR with constant domains from immunoglobulin Fc or λ subunits (Bass *et al.*, 1996). The recombinant gene was expressed in human embryonic kidney 293 cells. The expressed protein was a fully processed heterotetramer and the ability to bind insulin was similar to that of the full-length holoreceptor.

IGF-1 and insulin competitively cross-react with IGF-1R and IR. (Schäffer, 1994). Despite 45% overall amino acid homology, insulin and IGF-1 bind only weakly to each other's receptor. The affinity of each peptide for the non-cognate receptor is about 3 orders of magnitude lower than that for the cognate receptor. (Mynarcik, et al., 1997). The differences in binding affinities may be partly explained by the differences in amino acids and unique domains which contribute to unique tertiary structures of ligands. (Blakesley et al., 1996).

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Both insulin and IGF-1 are expressed as precursor proteins comprising, among other regions, contiguous A, B, and C peptide regions, with the C peptide being an intervening peptide connecting the A and B peptides. A mature insulin molecule is composed of the A and B chains connected by disulfide bonds, whereas the connecting C peptide has been removed during post-translational processing. IGF-1 retains its smaller Cpeptide as well as a small D extension at the C-terminal end of the A chain, making the mature IGF-1 slightly larger than insulin. (Blakesley, 1996). The C region of human insulin-like growth factor (IGF-1) appears to be required for high affinity binding to the type I IGF receptor. (Pietrzkowski et al., 1992). Specifically, tyrosine 31 located within this region appears to be essential for high affinity binding. Furthermore, deletion of the D domain of IGF-1 increased the affinity of the mutant IGF-1 for binding to the IR, while decreasing its affinity for the IGF-1R receptor. (Pietrzkowski et al., 1992). A further structural distinction between the two hormones is that, unlike insulin, IGF-1 has very weak self-association and does not hexamerize. (De Meyts, 1994).

The α -subunits, which contain the ligand binding region of the IR and IGF-1R, demonstrate between 47-67% overall amino acid homology. Three general domains have been reported for both receptors from sequence analysis of the α subunits, L1-Cys-rich-L2. The cysteine residues in the C-rich region are highly conserved between the two receptors; however, the cysteine-rich domains have only 48% overall amino acid homology.

Despite the similarities observed between these two receptors, the role of the domains in specific ligand binding are distinct. Through chimeric receptor studies, (domain swapping of the IR and IGF-1R α -subunits), researchers have reported that the sites of interaction of the ligands with their specific receptors differ. (Blakesley, *et al.*, 1996). For example, the cysteine-rich domain of the IGF-1R (amino acids 191-290) was determined to be essential for high-affinity IGF binding, but not insulin binding by introducing this IGF-1R region into the corresponding region of the IR

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(amino acids 198-300) and observing that the IR demonstrated high affinity binding of IGF-1 while maintaining high-affinity insulin binding. Conversely, when the corresponding region of the IR was introduced into the IGF-1R, the affinity for IGF-1 was not detectable while the affinity for insulin remained undisturbed.

A further distinction between the binding regions of the IR and IGF-1R is their differing dependence on the N-terminal and C-terminal regions. Both the N-terminal and C-terminal regions (located within the putative L1 and L2 domains) of the IR are important for high-affinity insulin binding but appear to have little effect on IGF-1 binding. Replacing residues in the Nterminus of IGF-1R (amino acids 1-62) with the corresponding residues of IR (amino acids 1-68) confers insulin-binding ability on IGF-1R. Within this region residues Phe-39, Arg-41 and Pro-42 are reported as major contributors to the interaction with insulin. (Williams et al., 1995). When these residues are introduced into the equivalent site of the IGF-1R, the affinity for insulin is markedly increased, whereas, substitution of these residues by alanine in the IR results in markedly decreased insulin affinity. Similarly, the region between amino acids 704-717 of the C-terminus of IR has been shown to play a major role in insulin specificity. Substitution of these residues with alanine also disrupts insulin binding. (Mynarcik et al., 1996).

Further studies of alanine scanning of the receptors suggest that insulin and IGF-1 may use some common contacts to bind to the IGF-1 receptor but that those contacts differ from those that insulin utilizes to bind to the insulin receptor. (Mynarcik *et al.*, 1997). Hence, the data in the literature has led one commentator to state that even though "the binding interfaces for insulin and IGF-1 on their respective receptors may be homologous within this interface the side chains which make actual contact and determine specificity may be quite different between the two ligand-receptor systems." (De Meyts, 1994).

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The identification of molecular structures having a high degree of specificity for one or the other receptor is important to developing efficacious and safe therapeutics. For example, a molecule developed as an insulin agonist should have little or no IGF-1 activity in order to avoid the mitogenic activity of IGF-1 and a potential for facilitating neoplastic growth.

It is therefore important to determine whether insulin and IGF-1 share common three-dimensional structures but which have sufficient differences to confer selectivity for their respective receptors. Similarly, it would be desirable to identify other molecular structures which mimic the active binding regions of insulin and/or IGF-1 and which impart selective agonist or antagonist activity.

Although certain proteins are important drugs, their use as therapeutics presents several difficult problems, including the high cost of production and formulation, administration usually via injection and limited stability in the bloodstream. Therefore, replacing proteins, including insulin or IGF-1, with small molecular weight drugs has received much attention. However, none of these efforts has resulted in finding a successful drug.

Peptides mimicking functions of protein hormones have been previously reported. Yanofsky *et al.* (1996) reports the isolation of a monomer peptide antagonistic to IL-1 with nanomolar affinity for the IL-1 receptor. This effort required construction and use of many phage displayed peptide libraries and sophisticated phage panning procedures.

Wrighton et al. (1996) and Livnah et al. (1996) reported dimer peptides that bind to the erythropoietin (EPO) receptor with full agonistic activity in vivo. These peptides are cyclical and have intra-peptide disulfide bonds; like the IL-1 receptor antagonist, they show no significant sequence identity to the natural ligand. Importantly, X-ray crystallography revealed that it was the spontaneous formation of non-covalent peptide homodimers which enabled the dimerization two EPO receptors.

Most recently, Cwirla et al. (1997) reported the identification of two families of peptides that bind to the human thrombopoietin (TPO) receptor

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and are competed by the binding of the natural TPO ligand. The peptide with the highest affinity, when dimerized by chemical means proved to be as potent an *in vivo* agonist as TPO, the natural ligand.

WO 96/04557 reports the use of peptides and antibodies which bind to active sites of biological targets and which are then used in competition assays to identify small molecules which are agonist or antagonists at the biological targets.

III. SUMMARY OF THE INVENTION

This invention relates to the identification of amino acid sequences that specifically recognize sites involved in IR and/or IGF-1R activation. Specific amino acid sequences are identified and their agonist or antagonist activity at IR or IGF-1R has been determined. Such sequences may be developed as potential therapeutics or as lead compounds to develop other more efficacious ones. In addition, these sequences may be used in high-throughput screens to identify and provide information on small molecules which bind at these sites and mimic or antagonize the functions of insulin or IGF-1. Furthermore, the peptide sequences provided by this invention can be used to design secondary peptide libraries, which can be used to identify sequence variants that increase or modulate the binding and/or activity of the original peptide at IR or IGF-1R.

In one aspect of this invention large numbers of peptides have been screened for their IR or IGF-1R binding and activity characteristics. Analysis of their amino acid sequences has identified certain consensus sequences which may be used themselves or as core sequences in larger amino acid sequences conferring upon them agonist or antagonist activity. At least ten generic amino acid sequences have been identified which bind IR and IGF-1R with varying degrees of agonist or antagonist activity depending on the specific sequence of the various peptides identified within each motif group. Also provided are amino or carboxyl terminal extensions capable of modifying the affinity and/or pharmacological activity of the consensus sequences when part of a larger amino acid sequence.

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The amino acid sequences of this invention which bind IR and/or IGF-1R include:

- a. $X_1 X_2 X_3 X_4 X_5$ wherein X_1, X_2, X_4 and X_5 are aromatic amino acids, and X_3 is any polar amino acid;
- b. $X_6 X_7 X_8 X_9 X_{10} X_{11} X_{12} X_{13}$ wherein X_6 and X_7 are aromatic amino acids, X_8 , X_9 , X_{11} and X_{12} are any amino acid, and X_{10} and X_{13} are hydrophobic amino acids;
 - c. X_{14} X_{15} X_{16} X_{17} X_{18} X_{19} X_{20} X_{21} wherein X_{14} , and X_{17} are hydrophobic amino acids, X_{15} , X_{16} , X_{18} and X_{19} are any amino acid, and X_{20} and X_{21} are aromatic amino acids.
 - d. X₂₂ X₂₃ X₂₄ X₂₅ X₂₆ X₂₇ X₂₈ X₂₉ X₃₀ X₃₁ X₃₂ X₃₃ X₃₄ X₃₅ X₃₆ X₃₇ X₃₈ X₃₉ X₄₀ X₄₁ wherein X₂₂, X₂₅, X₂₈, X₂₉, X₃₀, X₃₃, X₃₄, X₃₅, X₃₆, X₃₇, X₃₈, X₄₀, and X₄₁ are any amino acid, X₃₅ and X₃₇ may be any amino acid for binding to IR, whereas X₃₅ is preferably a hydrophobic amino acid and X₃₇ is preferably glycine for binding to IGF-1R and possess agonist or antagonist activity. X₂₃ and X₂₆ are hydrophobic amino acids. This sequence further comprises at least two cysteine residues, preferably at X₂₅ and X₄₀ X₃₁ and X₃₂ are small amino acids.
- e. X₄₂ X₄₃ X₄₄ X₄₅ X₄₆ X₄₇ X₄₈ X₄₉ X₅₀ X₅₁ X₅₂ X₅₃ X₅₄ X₅₅ X₅₆

 20 X₅₇ X₅₈ X₅₉ X₆₀ X₆₁ wherein X₄₂, X₄₃, X₄₄, X₄₅, X₅₃, X₅₅, X₅₆, X₅₈, X₆₀ and X₆₁ may be any amino acid, X₄₃, X₄₆, X₄₉, X₅₀, X₅₄ are hydrophobic amino acids, X₄₇ and X₅₉ are preferably cysteines, X₄₈ is a polar amino acid, and X₅₁, X₅₂ and X₅₇ are small amino acids.
- f. X₆₂ X₆₃ X₆₄ X₆₅ X₆₆ X₆₇ X₆₈ X₆₉ X₇₀ X₇₁ X₇₂ X₇₃ X₇₄ X₇₅ X₇₆

 25 X₇₇ X₇₈ X₇₉ X₈₀ X₈₁ wherein X₆₂, X₆₅, X₆₈, X₆₉, X₇₁, X₇₃, X₇₆, X₇₇, X₇₈, X₈₀, and X₈₁ may be any amino acid; X₆₃, X₇₀, X₇₄ are hydrophobic amino acids; X₆₄ is a polar amino acid, X₆₇ and X₇₅ are aromatic amino acids and X₇₂ and X₇₉ are preferably cysteines capable of forming a loop.
- g. $H X_{82} X_{83} X_{84} X_{85} X_{86} X_{87} X_{88} X_{89} X_{90} X_{91} X_{92}$ wherein X_{82} is proline or alanine, X_{83} is a small amino acid, X_{84} is selected from leucine,

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serine or threonine, X_{85} is a polar amino acid, X_{86} , X_{88} , X_{89} and X_{90} are any amino acid, and X_{87} , X_{91} and X_{92} are an aliphatic amino acid.

- h. $X_{104}\,X_{105}\,X_{106}\,X_{107}\,X_{108}\,X_{109}\,X_{110}\,X_{111}\,X_{112}\,X_{113}\,X_{114}$ wherein at least one of the amino acids of X_{106} through X_{111} , and preferably two, are tryptophan separated by three amino acids, and wherein at least one of X_{104} , X_{105} and X_{106} and at least one of X_{112} , X_{113} and X_{114} are cysteine; and
- i. an amino acid sequence comprising the sequence DYKDLCQSWGVRIGWLAGLCPKK.
- j. WX_{123} GYX_{124} WX_{125} X_{126} wherein X_{123} is selected from proline, glycine, serine, arginine, alanine or leucine, but more preferably proline; X_{124} is any amino acid, but preferably a charged or aromatic amino acid; X_{125} is a hydrophobic amino acid preferably leucine or phenylalanine, and most preferably leucine. X_{126} is any amino acid, but preferably a small amino acid.

In one embodiment, preferred amino acid sequences FYX₃ WF ("A6" motif) and FYX₈ X_9 L/IX₁₁ X_{12} L ("B6" motif) have been identified which competitively bind to sites on IR and IGF-1R and possess either agonist or antagonist activity. Surprisingly FYX₃WF which possesses agonist activity at IGF-1R, can possess agonist or antagonist activity at IR. Similarly, FY X₈ X_9 L/IX₁₁ X_{12} L, which is an antagonist at IGF-1R, possesses agonist activity at IR.

This invention also identifies at least two distinct binding sites on IR and IGF-1R based on the differing ability of certain of the peptides to compete with one another and insulin or IGF-1 for binding to IR and IGF-1R. Accordingly, this invention provides amino acid sequences which bind specifically to one or both sites of IR and/or IGF-1R. Furthermore, specific amino acid sequences are provided which have either agonist or antagonist characteristics based on their ability to bind to the specific sites of IR.

In another embodiment of this invention, amino acid sequences which bind to one or more sites of IR or IGF-1R may be covalently linked together

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to form multivalent ligands. These multivalent ligands are capable of forming complexes with a plurality of IR or IGF-1R. Either the same or different amino acid sequences may be covalently bound together to form homo- or heterocomplexes. Dimers of the same amino acid sequence, for example, may be used to form receptor complexes bound through the same corresponding sites. Alternatively, heterodimers may be used to bind to different sites on one receptor or to cause receptor complexing through different sites.

The present invention also provides assays for identifying compounds that mimic the binding characteristics of insulin. Such compounds may act as antagonists or agonists of insulin function in cell based assays.

This invention also provides amino acid sequences such as peptides and recombinant antibody variable regions (rVab) that inhibit binding of insulin to the insulin receptor. Such amino acid sequences and rVabs are used in the assays of the invention to identify compounds that mimic insulin.

This invention also provides kits for identifying compounds that bind to the insulin receptor. The invention further provides therapeutic compounds that bind the insulin receptor.

In another embodiment, this invention provides assays for identifying compounds which mimic the binding characteristics of IGF-1. Such compounds act as antagonists or agonists of IGF-1 hormone function in cell based assays.

The invention also provides amino acid sequences such as peptides and rVabs which inhibit binding of IGF-1 to IGF-1R. Such amino acid sequences and rVabs are used in the assays of the invention to identify compounds which mimic IGF-1.

Another embodiment of this invention is the nucleic acid sequences encoding the amino acid sequences of the invention. Also within the scope of this invention are vectors containing the nucleic acids and host cells which express the genes encoding the amino acid sequences which bind at IR or IGF-1R and possess agonist or antagonist activity.

It is an object of this invention to provide amino acid sequences which bind to active sites of IR and/or IGF-1R and to identify structural criteria for conferring agonist or antagonist activity at IR and/or IGF-1R.

It is a further object of this invention to provide specific amino acid sequences which possess agonist, partial agonist or antagonist activity at either IR or IGF-1R. Such amino acid sequences are potentially useful as therapeutics themselves or may be used to identify other molecules, especially small organic molecules, which possess agonist or antagonist activity at IR or IGF-1R.

Another object of this invention is to provide structural information derived from the amino acid sequences of this invention which may be used to construct other molecules possessing the desired activity at the relevant IR of IGF-1R binding site.

IV. BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-10G. Amino acid sequences comprising the motif of Formulas 1 through 10. Sequences were identified by panning peptide libraries against IGF-1R and/or IR. The amino acids are represented by their one-letter abbreviation. The ratios over background are determined by dividing the signal at 405 nm (E-Tag, IGF-1R, or IR) by the signal at 405 nm for non-fat milk. The IGF-1R/IR Ratio Comparison is determined by dividing the ratio of IGF-1R by the ratio of IR. The IR/IGF-1R Ratio Comparison is determined by dividing the ratio of IR by the ratio of IGF-1R.

The design of each library is shown in the first line in bold. In the design, symbol 'X' indicates a random position, an underlined amino acid indicates a doped position at the nucleotide level, and other positions are held constant. Additional abbreviations in the B6H library are: 'O' indicates an NGY codon where Y is C or T; 'J' indicates an RHR codon where R is A or G, and H is A, C, or T; and 'U' indicates an VVY codon where V is A, C, or G, and Y is C or T. The 'h' in the 20E2 libraries indicates an NTN codon.

Symbols in the listed sequences are: Q - TAG Stop; # -TAA Stop; * - TGA Stop; and ? - Unknown Amino Acid. It is believed that a W replaces

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the TGA Stop Codon when expressed. Except for the 20C, A6L, and B6L libraries, all libraries are designed with the short FLAG Epitope DYKD (Hopp *et al.*, 1988) at the N-terminus of the listed sequence and AAAGAP at the C-terminus. The 20C, A6L, and B6L libraries have the full length FLAG epitope DYKDDDDDK.

- Figure 1A: Formula 1 motif peptide sequences obtained from a random 40mer library panned against IR.
- Figure 1B: Formula 1 motif peptide sequence obtained from a random 40mer library panned against IGF-1R.
- Figure 1C: Formula 1 motif peptide sequences obtained from a random 20mer library panned against IR.
 - Figure 1D: Formula 1 motif peptide sequences obtained from a random 20mer library panned against IGF-1R.
- Figure 1E: Formula 1 motif peptide sequences obtained from a 21mer library constructed to contain X_{1.10}NFYDWFVX₁₈₋₂₁ (also referred to as "A6S") panned against IR.
 - Figure 1F: Formula 1 motif peptide sequences obtained from a 21mer library constructed to contain $X_{1-10}NFYDWFVX_{18-21}$ (also referred to as "A6S") panned against IGF-1R.
 - Figure 1G: Formula 1 motif peptide sequences obtained from a library constructed to contain variations outside the consensus core of the A6 peptide as indicated (referred to as "A6L") panned against IR.
 - Figure 1H: Formula 1 motif peptide sequences obtained from a library constructed to contain variations outside the consensus core of the A6 peptide as indicated (referred to as "A6L") panned against IGF-1R.
 - Figure 1I: Formula 1 motif peptide sequences obtained from a library constructed to contain variations in the consensus core of the E4D peptide (as indicated) panned against IR.
 - Figure 1J: Formula 1 motif peptide sequences obtained from a library constructed to contain variations in the consensus core of the E4D peptide (as indicated) panned against IGF-1R.

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Figure 1K: Formula 1 motif peptide sequences obtained from a library constructed using the sequence X₁₋₆FHENFYDWFVRQVSX₂₁₋₂₆ (H2C-A) panned against IR.

Figure 1L: Formula 1 motif peptide sequences obtained from a library constructed using the sequence X₁₋₆FHENFYDWFVRQVSX₂₁₋₂₆ (H2C-A) panned against IGF-1R.

Figure 1M: Formula 1 motif peptide sequences obtained from a library constructed using the sequence $X_{1-6}\underline{FH}XX\underline{FY}X\underline{WF}X_{16-21}$ (H2C-B) and panned against IR.

Figure 1N: Formula 1 motif peptide sequences obtained from a library constructed using the sequence $X_{1-6}\underline{FH}XX\underline{FY}X\underline{WF}X_{16-21}$ (H2C-B) and panned against IGF-1R.

Figure 10: Formula 1 motif peptide sequences obtained from other libraries panned against IR.

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Figure 2B: Formula 2 motif peptide sequences identified from a random 40mer library panned against IGF-1R.

Figure 2C: Formula 2 motif peptide sequences identified from a random 20mer library panned against IR.

Figure 2D: Formula 2 motif peptide sequences identified from a random 20mer library panned against IGF-1R.

Figure 2E: Formula 2 motif peptide sequences identified from a X_{1-4} C X_{6-20} library panned against IGF-1R.

Figure 2F: Formula 2 motif peptide sequences identified from a library constructed to contain variations outside the consensus core of the B6 peptide as indicated (referred to as "B6L") and panned against IR.

Figure 2G: Formula 2 motif peptide sequences identified from a library constructed to contain variations outside the consensus core of the B6 peptide as indicated (referred to as "B6L") and panned against IGF-1R.

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- Figure 2H: Formula 2 motif peptide sequences identified from a library constructed to contain a helix-turn-helix based on the B6 peptide as indicated (referred to as "B6H") and panned against IR.
- Figure 2I: Formula 2 motif peptide sequences identified from a library constructed to contain a helix-turn-helix based on the B6 peptide as indicated (referred to as "B6H") and panned against IGF-1R.
 - Figure 2J: Formula 2 motif peptide sequences identified from a library constructed to contain variations in the consensus core of B6 peptide as indicated (referred to as "B6C") and panned against IR.
 - Figure 2K: Formula 2 motif peptide sequences identified from a library constructed to contain variations in the consensus core of B6 peptide as indicated (referred to as "B6C") and panned against IGF-1R.
 - Figure 2L: Formula 2 motif peptide sequences identified from a library constructed using the sequence X₁₋₆FYDAIDQLVX₁₆₋₂₁ (20E2-A) panned against IR.
 - Figure 2M: Formula 2 motif peptide sequences identified from a library constructed using the sequence X₁₋₆FYDAIDQLVX₁₆₋₂₁ (20E2-A) panned against IGF-1R.
- Figure 2N: Formula 2 motif peptide sequences identified from a library constructed using the sequence X₁₋₆FYXXhXXhhX₁₆₋₂₁ (20E2-B) panned against IR.
 - Figure 2O: Formula 2 motif peptide sequences identified from a library constructed using the sequence X₁₋₆FYXXhXXhhX₁₆₋₂₁ (20E2-B) panned against IGF-1R.
- 25 Figure 2P: Formula 2 motif peptide sequences identified from a library constructed using the sequence X₁₋₆FYRYFXX<u>LL</u>X₁₆₋₂₁ (NNRP) panned against IR.
 - Figure 3A: Formula 3 motif peptide sequences identified from a random 20mer library panned against IGF-1R.
- Figure 3B: Formula 3 motif peptide sequences identified from a X_{1-} $_4CX_{6-20}$ library panned against IGF-1R.

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Figure 3C: Formula 3 motif peptide sequences identified from a library constructed using the sequence X₃LXXLXXYFX₁₂₋₁₇ (reverse B6; rB6) panned against IR.

Figure 3D: Formula 3 motif peptide sequences identified from a library constructed using the sequence X₃LXXLXXYFX₁₂₋₁₇ (reverse B6; rB6) panned against IGF-1R.

Figure 4A: Formula 4 motif peptide sequences identified from a random 20mer library panned against IR.

Figure 4B: Formula 4 motif peptide sequences identified from a library constructed to contain variations in the F8 peptide as indicated (15% dope; referred to as "F815") panned against IR.

Figure 4C: Formula 4 motif peptide sequences identified from a library constructed to contain variations in the F8 peptide as indicated (15% dope; referred to as "F815") panned against IGF-1R.

Figure 4D: Formula 4 motif peptide sequences identified from a library constructed to contain variations in the F8 peptide as indicated (20% dope; referred to as "F820") panned against IR.

Figure 4E: Formula 4 motif peptide sequences identified from other libraries panned against IR.

Figure 5: Formula 5 motif peptide sequences identified from a library constructed to contain variations in the F8 peptide as indicated (15% dope; referred to as "F815") panned against IGF-1R.

Figure 6A: Formula 6 motif peptide sequences identified from a random 20mer library and panned against IR.

Figure 6B: Formula 6 motif peptide sequences identified from a library constructed to contain variations in the D8 peptide as indicated (15% dope; referred to as "D815") panned against IR.

Figure 6C: Formula 6 motif peptide sequences identified from a library constructed to contain variations in the D8 peptide as indicated (20% dope; referred to as "D820") panned against IR.

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Figure 6D: Formula 6 motif peptide sequences identified from a library constructed to contain variations in the D8 peptide as indicated (20% dope; referred to as "D820") panned against IGF-1R.

Figure 6E: Formula 6 motif peptide sequences identified from other 5 libraries panned against IR.

Figure 7: Formula 7 motif peptide sequences.

Figure 8: Formula 8 motif peptide sequences identified from a commercial phage display peptide library and synthetic sequences. Small letters denote D-amino acids. Unnatural amino acids are denoted with a 3-letter abbreviation in certain sequences. K_d values greater than 2 x 10^{-5} are approximate.

Figure 9A: Formula 9 motif peptide sequences identified from a library constructed to contain variations in the H5 peptide as indicated (referred to as "H5") panned against IGF-1R.

Figure 9B: Formula 9 motif peptide sequences identified from a library constructed to contain variations in the JBA5 peptide as indicated (referred to as "JBA5") panned against IGF-1R.

Figure 9C: Formula 9 motif peptide sequences identified from a library constructed to contain variations in the JBA5 peptide as indicated (referred to as "JBA5") panned against IR.

Figure 10A: Formula 10 motif peptide sequences identified from random 20mer libraries panned against IGF-1R.

Figure 10B: Formula 10 motif peptide sequences identified from random 20mer libraries panned against IR.

Figure 10C: Miscellaneous peptide sequences identified from a random 20mer library panned against IR.

Figure 10D: Miscellaneous peptide sequences identified from a random 40mer library panned against IR.

Figure 10E: Miscellaneous peptide sequences identified from a random 20mer library panned against IGF-1R.

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Figure 10F: Miscellaneous peptide sequences identified from a X_{1-} $_4CX_{6-20}$ and panned against IGF-1R.

Figure 10G: Miscellaneous peptide sequences identified from a library constructed to contain variations of the F8 peptide as indicated (F815) panned against IGF-1R.

Figure 10H: Miscellaneous peptide sequences identified from a library constructed to contain variations in the F8A11 peptide as indicated (referred to as "NNKH") panned against IR.

Figure 10I: Miscellaneous peptide sequences identified from a library constructed to contain variations in the F8A11 peptide as indicated (referred to as "NNKH") panned against IGF-1R.

Figure 11A: Summary of specific representative amino acid sequences from Formulas 1 through 11.

Figure 11B: Summary of specific representative amino acid sequences from Formulas 1 through 11.

Figure 12: Illustration of helix wheels applied to Formula 2 and 3 motifs.

Figure 13: Illustration of 2 binding site domains on IR based on competition data.

Figure 14: Dissociation of 20E2 peptide from IGF-1R in the presence of buffer (filled circle), 30 μM IGF-1 (open circle), 100 μM H2C (filled square), 100 μM 20E2 (filled triangle), 100 μM D8 (B12; open square), 100 μM C1 (filled, inverted triangle) and 100 μM RPG (filled diamond).

Figure 15: Schematic illustration of potential binding schemes to the multiple binding sites on IR.

Figure 16: Schematic diagram of the phage-displayed peptide library. The peptide is displayed as a protein fusion to the N-terminus of gene *III* encoding the minor coat protein of the phage.

Figure 17: BIAcore analysis of competition binding between IR and 30 MBP fusion H2C-9-H2C, H2C and H2C-3-H2C.

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Figure 18: Sequence alignments of Class I and Class II peptides.

The Class I peptides have been shown to be IGF-1R antagonists, while the Class II peptides are IGF-1R agonists.

Figure 19: DNA sequences of the frameshifted clones.

Figures 20A and 20B: Results of the phage ELISA for binding to IGF-1R. Wells were coated with 100 ng/well IGF-1R and blocked. Competitor, the IGF-1 native ligand, was present prior (1 h) and during the phage incubation (1 h). Phage were detected with HRP-anti M13 phage antibody and reported as OD₄₀₅ as described. Total Binding is shown in Figure 20A and Percent Inhibition is shown in Figure 20B.

Figure 21: Sequences of the designed IGF-1R-specific synthetic peptides.

Figure 22: Assay results showing that Motif 2 peptides (5.1 and 5.2) antagonize the effects of IGF-1 on IGF-1R⁺ cells.

Figure 23: Assay results showing that Motif 1 peptides (5.3 and 5.4) stimulate growth of IGF-1R $^+$ cells. Cells expressing human IGF-1R (30,000 cells per well) were incubated with the 5.4 peptide for 42 h at 37 $^\circ$ C. Experiments were done in triplicate. Background signal A₄₅₀=0.15. Proliferation was measured using WST-1 reagent (Boehringer Mannheim Biochemicals/Roche Molecular Biochemicals, Indianapolis, IN).

Figures 24A and 24B: Demonstration of binding of peptide 5.1 to IGF-1R using BIAcore. Figure 24A: Binding as a function of the peptide concentration. Figure 24B: Inhibition of IGF-1 binding by peptide 5.1. RU – refractive units.

Figures 25A and 25B: Design of the secondary phage library A6L based on the Class II peptide sequences. Figure 25A: Design of the sequence of the gene. Underlined residues indicate positions mutated to optimize the codons for expression in *E. coli*. Figure 25B: Synthetic oligonucleotide for the A6L secondary library. Underlined residues were doped in the chemical DNA synthesis. Definitions of mixes (all mixes are equimolar) are as follows: N = A, C, G, or T; K = G or T. Nucleosides were

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premixed in the bottle (and not line mixed) to improve the accuracy of nucleoside mixes. The sequence of the FLAG epitope is shown in bold.

Figures 26A and 26B: Design of the secondary phage library A6S based on the Motif 1 peptide sequences. Figure 26A: Sequence design for the A6S secondary phage library. Figure 26B: Synthetic oligonucleotide for the A6L secondary library. Definitions of mixes (all mixes are equimolar) are as follows: N = A, C, G, or T; K = G or T. Nucleosides were premixed in the bottle (and not line mixed) to improve the accuracy of nucleoside mixes. The sequence of the FLAG epitope is shown in bold.

Figure 27: Sequences of the five H5-like peptides that show agonistic activity toward IGF-1R. The C-terminal lysine contains a biotin moiety linked to the amino group of the side chain.

Figure 28: Listing of amino acid sequences obtained from panning with the A6S library.

Figure 29: Listing of amino acid sequences obtained from panning with the H5 secondary phage library.

Figure 30: Schematic of the genomic rVab library.

Figure 31: Listing of the V_{H} , kappa and lambda genes used to assemble the rVab antibody library for IGF-1R binders.

Figure 32. Schematic of the assembly of the single-chain IGF-I and insulin antibody libraries from restriction fragments.

Figure 33: Sequences of the restriction fragments used to assemble the rVab libraries.

Figure 34: Nucleotide sequence of the gene encoding the 43G7 rVab specific for IGF-1R. The predicted amino acid sequence of the rVab is shown below the nucleic acid sequence.

Figure 35: Nucleotide sequence of the gene encoding the 1G2P rVab specific for IGF-1R. The predicted amino acid sequence of the rVab is shown below the nucleic acid sequence.

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Figure 36: Nucleotide sequence of gene encoding the 39F7 rVab specific for IGF-1R. The predicted amino acid sequence of the rVab is shown below the nucleic acid sequence.

Figure 37: Nucleotide sequence of gene encoding the M100 rVab specific for IGF-1R. The predicted protein sequence of the rVab is shown below the nucleic acid sequence.

Figure 38: Nucleotide sequence of gene encoding the 46A7 rVab specific for IGF-1R. The predicted protein sequence of the rVab is shown below the nucleic acid sequence.

Figure 39: Nucleotide sequence of gene encoding the 49E8 rVab specific for IGF-1R. The predicted protein sequence of the rVab is shown below the nucleic acid sequence.

Figure 40: Assay results demonstrating the binding of soluble forms of three rVabs to IGF-1R.

Figure 41: Assay results showing that the 43G7 rVab stimulates growth of IGF-1R⁺ cells.

Figure 42: Assay results showing that the stimulation by rVab 43G7 is antagonized by the 1G2P, 49E8, and 46A7 rVabs. The assay was done on IGF-1R⁺ cells.

Figure 43: Eu-based fluorescence assay results showing that the binding of peptide 5.1 to IGF-1R can be competed by the IGF-1 ligand.

Figure 44: Results of the time-resolved fluorescence assay showing that the binding of 43G7 rVab to IGF-1R is effectively competed by IGF-1.

Figure 45: Eu-based fluorescence assay showing that the binding of the B6 peptide to IGF-1R is effectively competed by the 43G7 rVab.

Figures 46A-46D: Results of the Eu-based fluorescence assay showing that the binding of the europium-labeled 43G7 rVab to IGF-1R is effectively competed by selected scAbs specific for IGF-1R.

Figure 47: Biopanning results and sequence alignments of Group 1 of IR-binding peptides. The number of sequences found is indicated on the right side of the figure together with data on the phage binding to either IR or

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IGF-1R receptor. Absorbance signals are indicated by: ++++, >30X over background; +++, 15-30X; ++, 5-15X; +, 2-5X; and 0, <2X.

Figure 48: Biopanning results and sequence alignments of Groups 2 through 7 of IR-binding peptides. The number of sequences found is indicated on the right side of the figure together with data on the phage binding to either IR or IGF-1R receptor.

Figure 49A-49D: Dose response curve of D118 peptide (Formula 2 motif) stimulated increase of ³H-glucose into mouse adipocytes.

Figures 50A-50D: Titration of the synthetic peptides C1 (Figures 50A, 50C) or B6 (Figures 50B, 50D) against constant concentration of phage bound to IR (Figures 50A, 50B) or IGF-1R (Figures 50C, 50D). Phage are represented by: open circle – 20D3; open square – 20A4; open triangle – 20E2; open diamond – F2; filled circle – F8; and filled square – D8.

Figure 51A-51D: Titration of the IGF-1R synthetic peptides against constant concentration of phage. Symbols for the peptides are: open circles – H2; filled circles – H2C; open square – C1; filled square – C1C; open triangle – D2C; filled triangle – E4; open diamond – A6; and filled diamond p53.

Figure 52A-52D: Hill plot analysis of phage clones. The detailed data are provided in Table 7. Symbols are the same as in Figure 51.

Figure 53: Competition between the insulin and the IR-binding phage. The results for seven different groups (categories) of phage binders are shown.

Figure 54: Titration of the synthetic peptide 20A4 against constant concentration of phage. Phage binding to IR are represented by: open circle – 20D3; filled circle B8; open square – 20A4; filled square – D8; open up triangle – 20E2; open down triangle – D10; filled down triangle – A2; open diamond – F2; filled diamond – E8; and cross-filled circle – F8.

Figure 55: A schematic drawing for the construction of protein fusions of the maltose binding protein and peptides from phage libraries.

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Figure 56A-56C: Insulin Receptor Competition ELISA using MBP-Peptide Fusion Proteins. Figure 54A. Competition with fusion proteins containing cysteine residues. The hatched bars indicate value is ≤ 54 % control value. Figure 54B. Competition with fusion proteins containing the consensus sequence. The notation, c-c, indicates phage displayed peptides with cysteine residues. Figure 54C. Competition with fusion protein containing a control peptide.

Figure 57: Nucleotide and predicted amino acid sequence of the gene encoding the 6f6 rVab that binds to IR.

Figure 58: Nucleotide and predicted amino acid sequence of the gene encoding the 14c8 rVab that binds to IR.

Figure 59: Comparison of the VH CDR3 sequences of different rVabs that bind to IR, and competitions of these rVabs and insulin for binding to IR.

Figure 60: Biological response of insulin, rVab 12h10, and rVab 13h9 in 32D cells expressing or not expressing IR.

Figure 61: Competition of rVab 6f6 and insulin for binding to IR.

Figure 62: Competition of rVab 6f6 and IGF-1 for binding to IR.

Figure 63: Competition of synthetic peptides and soluble rVab antibodies for binding of biotinylated peptides to insulin receptor. Synthetic peptides or soluble rVab at indicated concentrations were incubated with biotinylated peptides overnight using the heterogeneous TRFA.

Figure 64: Binding of C1 to IR and IGF-1R.

Figure 65: Competition of peptides for binding to IR.

Figure 66: H2C competition for b-peptide binding to IR. Biotinylated peptides at indicated concentrations were competed by increasing concentrations of H2C for binding to IR using the heterogeneous TRFA.

Figure 67: C1C competition for b-C1 binding to IR. Biotinylated C1 peptide at 0.3 μ M was competed by increasing concentrations of C1C for binding to IR using the heterogeneous TRFA.

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Figure 68: Competition of peptides for binding of rVab 12H10 to insulin receptor. Synthetic peptides at indicated concentration were incubated with rVab 12H10 overnight using the heterogeneous TRFA.

Figure 69: Competition of MBP-peptide fusion proteins to rVab 12H10 binding to insulin receptor. Four MBP-peptides fusion proteins at indicated concentrations were incubated with rVab 12H10 overnight using the heterogeneous TRFA.

Figures 70A-70N: Peptide binding displacement curves showing the displacement of ¹²⁵insulin or ¹²⁵IGF-1 from HIR or HIGF-1R in the presence of various peptides.

Figures 71A-71Z; 71A2-71Z2; 71A3-71B3: Concentration dependent modulation of ³H-glucose into adipocytes by various peptides. Formula 1 motif peptide responses are shown in Figures 71A-71V; 71A2-71J2; Formula 9 motif peptide response is shown in Figures 71W-71Z; Formula 2 motif peptide response is shown in Figures 71K2-71L2; Miscellaneous peptide motif 10 peptide responses are shown in Figures 71M2-71P2; Formula 6 motif peptide response is shown in Figure 71Q2-Figure 71R2; and Formula 4 motif peptide response is shown in Figure 71S2-Figure 71W2. Formula 1 and Formula 2 motif peptide response is shown in Figure 71S3. Fusion peptide S291 response is shown in Figure 71B3.

Figures 72A and 72B: Competition of Site 1(Figure 72B) and Site 2 (Figure 72A) phage displayed peptides with recombinant cleaved dipeptides.

Figure 73: Competition of IGF-1R, peptide H2C (D117), peptide C1 (D112), and peptide RP6 (20C-3-G3-IGFR) in a homogeneous fluorescent-resonance energy transfer assay based on the binding of IGF-1R to peptide 20E2 (D118).

Figure 74: Stimulation of IR autophosphorylation *in vivo* by MBP-fusion peptides.

V. DETAILED DESCRIPTION OF THE INVENTION

This invention relates to amino acid sequences comprising motifs which bind to the IGF-1 receptor (IGF-1R) and/or the insulin receptor (IR).

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In addition to binding to IR and IGF-1R, the amino acid sequences also possess either agonist, partial agonist or antagonist activity at one or both of these receptors. Based on the differing regions of IR and IGF-1R which are reported to be important for binding and activity, this invention surprisingly provides amino acid sequences which define common binding motifs on IR and IGF-1R which are capable of conferring agonist and/or antagonist activity at these receptors. In addition, this invention identifies multiple binding sites (Sites 1 and 2) on IR and IGF-1R which appear to be allosterically coupled.

Although capable of binding to IR and/or IGF-1R at sites which participate in conferring agonist or antagonist activity, the amino acid sequences are neither based on insulin or IGF-1 native sequences, nor do they reflect an obvious homology to any such sequence.

The amino acid sequences of the invention may be peptides, polypeptides, or proteins. These terms as used herein should not be considered limiting with respect to the size of the various amino acid sequences referred to herein and which are encompassed within this invention. Thus, any amino acid sequence comprising at least one of the IR or IGF-1R binding motifs disclosed herein, and which binds to one of the receptors is within the scope of this invention. In preferred embodiments, the amino acid sequences confer insulin or IGF agonist or antagonist activity. The amino acid sequences of the invention are typically artificial, i.e. non-naturally occurring peptides or polypeptides. Amino acid sequences useful in the invention may be obtained through various means such as chemical synthesis, phage display, cleavage of proteins or polypeptides into fragments, or by any means which amino acid sequences of sufficient length to possess binding ability may be made or obtained.

The amino acid sequences provided by this invention should have an affinity for IR or IGF-1R sufficient to provide adequate binding for the intended purpose. Thus, for use as a therapeutic, the peptide, polypeptide or protein provided by this invention should have an affinity (K_d) of between

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about 10⁻⁷ to about 10⁻¹⁵ M. More preferably the affinity is 10⁻⁸ to about 10⁻¹² M. Most preferably, the affinity is 10⁻⁹ to about 10⁻¹¹ M. For use as a reagent in a competitive binding assay to identify other ligands, the amino acid sequence preferably has affinity for the receptor of between about 10⁻⁵ to about 10⁻¹² M.

A further consideration in identifying peptides provided by this invention for use as therapeutics is the relative activity at either IR or IGF-IR. Thus, a peptide which has efficacy at IR and clinically insignificant activity of IGF-IR may be a useful therapeutic even though such a peptide may bind IGF-IR with relatively high affinity.

At least ten different binding motifs have been identified which bind to active sites on IR; at least four of these also bind to IGF-1R. The binding motifs are defined based on the analysis of several different amino acid sequences and analyzing the frequency that particular amino acids or types of amino acids occur at a particular position of the amino acid sequence.

For the purposes of this invention, the amino acids are grouped as follows: amino acids possessing alcohol groups are serine (S) and threonine (T). Aliphatic amino acids are isoleucine (I), leucine (L), valine (V), and methionine (M). Aromatic amino acids are phenylalanine (F), histidine (H), tryptophan (W), and tyrosine (Y). Hydrophobic amino acids are alanine (A), cysteine (C), phenylalanine (F), glycine (G), histidine (H), isoleucine (I), lysine (L), methionine (M), arginine (R), threonine (T), valine (V), tryptophan (W), and tyrosine (Y). Negative amino acids are aspartic acid (D) and glutamic acid (E). The following amino acids are polar amino acids: cysteine (C), aspartic acid (D), glutamic acid (E), histidine (H), lysine (K), asparagine (N), glutamine (Q), arginine (R), serine (S), and threonine (T). Positive amino acids are histidine (H), lysine (K), and arginine (R). Small amino acids are alanine (A), cysteine (C), aspartic acid (D), glycine (G), asparagine (N), proline (P), serine (S), threonine (T), and valine (V). Very small amino acids are alanine (A), glycine (G) and serine (S). Amino acids likely to be involved in a turn formation are alanine (A), cysteine (C),

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aspartic acid (D), glutamic acid (E), glycine (G), histidine (H), lysine (K), asparagine (N), glutamine (Q), arginine (R), serine (S), proline (P), and threonine (T).

The amino acids within each of these defined groups may be substituted for each other in the motifs described below, subject to the specific preferences stated herein. In addition, synthetic or non-naturally occurring amino acids may also be used in accordance with this invention.

Also included within the scope of this invention are amino acid sequences containing substitutions, additions, or deletions based on the teachings disclosed herein and which bind to IR or IGF-1R with the same or altered affinity. For example, amino acid residues located at the carboxy and amino terminal regions of the consensus motifs described below, which amino acid residues are not associated with a strong preference for a particular amino acid, may optionally be deleted providing for truncated sequences. Certain amino acids such as lysine which promote the stability of the amino acids sequences may be deleted depending on the use of the sequence, as for example, expression of the sequence as part of a larger sequence which is soluble, or linked to a solid support.

Peptides that bind to IGF-1R, and methods and kits for identifying such peptides, have been disclosed by Beasley et al., U.S. Application Serial No. 09/146,127, filed September 2, 1998, which is incorporated by reference in its entirety.

A. Consensus Motifs

The following motifs have been identified as conferring binding activity to IR and/or IGF-1R:

1. $X_1X_2X_3X_4X_5$ (Formula 1, the A6 motif) wherein X_1 , X_2 , X_4 and X_5 are aromatic amino acids, preferably, phenylalanine or tyrosine. Most preferably, X_1 and X_5 are phenylalanine and X_2 is tyrosine. X_3 may be any small polar amino acid, but is preferably selected from aspartic acid, glutamic acid, glycine, or serine, and is most preferably aspartic acid or

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glutamic acid. X₄ is most preferably tryptophan, tyrosine, or phenylalanine and most preferably tryptophan. Particularly preferred embodiments of the A6 motif are FYDWF and FYEWF. The A6 motif possesses agonist activity at IGF-1R, but agonist or antagonist activity at IR depending on the identity of amino acids flanking A6. See Figure 11A. Two amino acid sequences comprising the A6 motif possess agonist activity at IR are FHENFYDWFVRQVSKK (D117; H2C) and GRVDWLQRNANFYDWFVAELG-NH₂ (S175). Nonlimiting examples of Formula 1 amino acid sequences are shown in Figures 1A-1O.

2. X₆X₇X₈X₉X₁₀X₁₁X₁₂X₁₃ (Formula 2, the B6 motif) wherein X₆ and X₇ are aromatic amino acids, preferably, phenylalanine or tyrosine. Most preferably, X₆ is phenylalanine and X₇ is tyrosine. X₈, X₉, X₁₁ and X₁₂ may be any amino acid. X₁₀ and X₁₃ are hydrophobic amino acids, preferably leucine, isoleucine, phenylalanine, tryptophan or methionine, but more preferably leucine or isoleucine. X₁₀ is most preferably isoleucine for binding to IR and leucine for binding to IGF-1R. X₁₃ is most preferably leucine. Amino acid sequences of Formula 2 may function as an antagonist at the IGF-1R, or as an agonist at the IR. Preferred consensus sequences of the Formula 2 motif are FYX₈ X₉ L X₁₁ X₁₂L, FYX₈ X₉ IX₁₁ X₁₂L FYX₈ AIX₁₁ X₁₂L, and FYX₈ YFX₁₁ X₁₂L.

Another Formula 2 motif for use with this invention comprises FYX₈ YFX₁₁ X₁₂ L and is shown as Formula 2A ("NNRP") below: X₁₁₅ X₁₁₆ X₁₁₇ X₁₁₈ FY X₈ YF X₁₁ X₁₂ L X₁₁₉ X₁₂₀ X₁₂₁ X₁₂₂, wherein X₁₁₅-X₁₁₈ and X₁₁₈-X₁₂₂ may be any amino acid which allows for binding to IR or IGF-1R. X₁₁₅ is preferably selected from the group consisting of tryptophan, glycine, aspartic acid, glutamic acid and arginine. Aspartic acid, glutamic acid, glycine, and arginine are more preferred. Tryptophan is most preferred. The preference for tryptophan is based on its presence in clones at a frequency three to five fold higher than that expected over chance for a random substitution, whereas aspartic acid,

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glutamic acid and arginine are present about two fold over the frequency expected for random substitution.

 X_{116} preferably is an amino acid selected from the group consisting of aspartic acid, histidine, glycine, and asparagine. X_{117} and X_{118} are preferably glycine, aspartic acid, glutamic acid, asparagine or alanine. More preferably X_{117} is glycine, aspartic acid, glutamic acid and asparagine whereas X_{118} is more preferably glycine, aspartic acid, glutamic acid or alanine.

X₈ when present in the Formula 2A motif is preferably arginine, glycine, glutamic acid, or serine.

X₁₁ when present in the Formula 2A motif is preferably glutamic acid, asparagine, glutamine, or tryptophan, but most preferably glutamic acid.

 X_{12} when present in the Formula 2A motif is preferably aspartic acid, glutamic acid, glycine, lysine or glutamine, but most preferably aspartic acid.

X₁₁₉ is preferably glutamic acid, glycine, glutamine, aspartic acid or alanine, but most preferably glutamic acid.

 X_{120} is preferably glutamic acid, aspartic acid, glycine or glutamine, but most preferably glutamic acid.

 X_{121} is preferably tryptophan, tyrosine, glutamic acid, phenylalanine, histidine, or aspartic acid, but most preferably tryptophan or tyrosine.

 X_{122} is preferably glutamic acid, aspartic acid or glycine; but most preferably glutamic acid.

Preferred amino acid residue are identified based on their frequency in clones over two fold over that expected for a random event, whereas the more preferred sequences occur about 3-5 times as frequently as expected.

Nonlimiting examples of amino acid sequences having the Formula 2 and 2A motifs are described in Figures 2A-2P.

3. $X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}$ (Formula 3, reverse B6, revB6), wherein X_{14} and X_{17} are hydrophobic amino acids; X_{14} , X_{17} are preferably leucine, isoleucine, and valine, but most preferably leucine; X_{15} , X_{16} , X_{18} and X_{19} may be any amino acid; X_{20} is an aromatic amino acid, preferably

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tyrosine or histidine, but most preferably tyrosine; and X_{21} is an aromatic amino acid, but preferably phenylalanine or tyrosine, and most preferably phenylalanine. For use as an IGF-1R binding ligand, an aromatic amino acid is strongly preferred at X_{18} . See Figures 3A-3D for nonlimiting examples of Formula 3 amino acid sequences.

4. X₂₂X₂₃X₂₄X₂₅X₂₆X₂₇X₂₈X₂₉X₃₀X₃₁X₃₂X₃₃X₃₄X₃₅X₃₆X₃₇X₃₈X₃₉X₄₀X₄₁ (Formula 4, "F8") wherein X₂₂, X₂₅, X₂₆, X₂₈, X₂₉, X₃₀, X₃₃, X₃₄, X₃₅, X₃₆, X₃₇, X_{38} , X_{40} , and X_{41} are any amino acid. X_{35} and X_{37} may be any amino acid when the F8 motif is used as an IR binding ligand or as a component of an IR binding ligand, however for use as an IGF-1R binding ligand, glycine is strongly preferred at X₃₇ and a hydrophobic amino acid, particularly, leucine, is preferred at X₃₅. X₂₃ is a hydrophobic amino acid. Methionine, valine, leucine or isoleucine are preferred amino acids for X23, however, leucine which is most preferred for preparation of an IGF-1R binding ligand is especially preferred for preparation of an IR binding ligand. At least one cysteine is located at X₂₄ through X₂₇, and one at X₃₉ or X₄₀. Together the cysteines are capable of forming a cysteine cross-link to create a looped amino acid sequence. In addition, although a spacing of 14 amino acids in between the two cysteine residues is preferred, other spacings may also be used provided binding to IGF-1R or IR is maintained. Accordingly, other amino acids may be substituted for the cysteines at positions X₂₄ and X₃₉ if the cysteines occupy other positions. In one embodiment, for example, the cysteine at position X₂₄ may occur at position X₂₇ which will produce a smaller loop provided that the cysteine is maintained at position X₃₉. These smaller looped peptides are described herein as Formula 5, infra. X₂₇ is any polar amino acid, but is preferably selected from glutamic acid, glutamine, aspartic acid, asparagine, or as discussed above cysteine. The presence of glutamic acid at position X₂₇ decreases binding to IR but has less of an effect on binding to IGF-1R. X₃₁ is any aromatic amino acid and X₃₂ is any small amino acid. For binding to IGF-1R, glycine or serine are preferred at position X₃₁, however, tryptophan is highly preferred for binding to IR. At

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position X_{32} , glycine is preferred for both IGF-1R and IR binding. X_{36} is an aromatic amino acid. A preferred consensus sequence for F8 is X_{22} LC X_{25} X_{26} E X_{28} X_{29} X_{30} WG X_{33} X_{34} X_{35} X_{36} X_{37} X_{38} C X_{40} X_{41} whereas the amino acids are defined above. A more preferred F8 sequence is

- 5 HLCVLEELFWGASLFGYCSG ("F8"). Amino acid sequences comprising the F8 sequence motif preferably bind to IR over IGF-1R. Figures 4A-4E list nonlimiting examples of Formula 4 amino acid sequences.
- 5. X₄₂ X₄₃ X₄₄ X₄₅ X₄₆ X₄₇ X₄₈ X₄₉ X₅₀ X₅₁ X₅₂ X₅₃ X₅₄ X₅₅ X₅₆X ₅₇ X₅₈ X₅₉ X₆₀ X₆₁ ("mini F8", Formula 5) wherein X₄₂, X₄₃, X₄₄, X₄₅, X₅₃, X₅₅, X₅₆, X₅₆, X₅₈, X₆₀ and X₆₁ are any amino acid. X₄₃, X₄₆, X₄₉, X₅₀ and X₅₄ are hydrophobic amino acids, however, X₄₃ and X₄₆ are preferably leucine, whereas X₅₀ is preferably phenylalanine or tyrosine but most preferably phenylalanine. X₄₇ and X₅₉ are cysteines. X₄₈ is preferably a polar amino acid, i.e. aspartic acid or glutamic acid, but most preferably glutamic acid.
 Use of the small amino acid at position 54 may confer IGF-1R specificity. X₅₁, X₅₂ and X₅₇ are small amino acids, preferably glycine. A preferred consensus sequence for mini F8 is X₄₂ X₄₃ X₄₄ X₄₅ LCEX₄₉ FGGX₅₃ X₅₄ X₅₅ X₅₆ GX₅₈ CX₆₀ X₆₁. Amino acid sequences comprising the sequence of Formula 5 preferably bind to IGF-1R or IR. Nonlimiting examples of

Formula 5 amino acid sequences are described in Figure 5.

6. $X_{62} X_{63} X_{64} X_{65} X_{66} X_{67} X_{68} X_{69} X_{70} X_{71} X_{72} X_{73} X_{74} X_{75} X_{76} X_{77} X_{78} X_{79} X_{80} X_{81}$ (Formula 6, "D8") wherein X_{62} , X_{65} , X_{68} , X_{69} , X_{71} , X_{73} , X_{76} , X_{77} , X_{78} , X_{80} and X_{81} may be any amino acid. X_{66} may also be any amino acid, however, there is a strong preference for glutamic acid. Substitution of X_{66} with glutamine or valine may result in attenuation of binding. X_{63} , X_{70} , and X_{74} are hydrophobic amino acids. X_{63} is preferably leucine, isoleucine, methionine, or valine, but most preferably leucine. X_{70} and X_{74} are preferably valine, isoleucine, leucine, or methionine. X_{74} is most preferably valine. X_{64} is a polar amino acid, more preferably aspartic acid or glutamic acid, and most preferably glutamic acid. X_{67} and X_{75} are aromatic amino acids. Whereas tryptophan is highly preferred at X_{67} , X_{75} is preferably

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tyrosine or tryptophan but most preferably tyrosine. X_{72} and X_{79} are cysteines which again are believed to form a loop which position amino acid may be altered by shifting the cysteines in the amino acid sequence. D8 is most useful as an amino acid sequence having a preference for binding to IR as only a few D8 sequences capable of binding to IGF-1R over background have been detected. A preferred sequence for binding to IR is $X_{62} L X_{64} X_{65} X_{66} W X_{68} X_{69} X_{70} X_{71} C X_{73} X_{74} X_{75} X_{76} X_{77} X_{78} C X_{80} X_{81}$. Nonlimiting examples of Formula 6 amino acid sequences are described in Figures 6A-6E.

- 7. HX₈₂, X₈₃, X₈₄ X₈₅ X₈₆ X₈₇ X₈₈ X₈₉ X₉₀ X₉₁ X₉₂ (Formula 7) wherein X₈₂ is proline or alanine but most preferably proline; X₈₃ is a small amino acid more preferably proline, serine or threonine and most preferably proline; X₈₄ is selected from leucine, serine or threonine but most preferably leucine; X₈₅ is a polar amino acid preferably glutamic acid, serine, lysine or asparagine but more preferably serine; X₈₆ may be any amino acid but is preferably a polar amino acid such as histidine, glutamic acid, aspartic acid, or glutamine; X₈₇ is an aliphatic amino acid preferably leucine, methionine or isoleucine and most preferably leucine; amino acid X₈₈, X₈₉ and X₉₀ may be any amino acids; X₉₁ is an aliphatic amino acid with a strong preference for leucine as is X₉₂. Phenylalanine may also be used at position 92. A preferred consensus sequence of Formula 7 is HPPLSX₈₆LX₈₈X₈₉X₉₀LL. The Formula 7 motif binds to IR with little or no binding to IGF-1R. Nonlimiting examples of Formula 7 amino acid sequences are described in Figure 7.
- 8. Another sequence is X₁₀₄, X₁₀₅ X₁₀₆ X₁₀₇ X₁₀₈ X₁₀₉ X₁₁₀ X₁₁₁ X₁₁₂ X₁₁₃ X₁₁₄. (Formula 8) which comprises eleven amino acids wherein at least one, and preferably two of the amino acids of X₁₀₆ through X₁₁₁ are tryptophan. In addition, it is also preferred that when two tryptophan amino acids are present in the sequence they are separated by three amino acids, which are preferably, in sequential order proline, threonine and tyrosine with proline being adjacent to the tryptophan at the amino terminal end.

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Accordingly, the most preferred sequence for X₁₀₇ X₁₀₈ X₁₀₉ X₁₁₀ X₁₁₁ is WPTYW. At least one of the three amino acids on the amino terminal (X₁₀₄, X₁₀₅ X₁₀₆) and at least one of the amino acids carboxy terminal (X₁₁₂ X₁₁₃ X₁₁₄) ends immediately flanking X₁₀₇-X₁₁₁ are preferably a cysteine residue, most preferably at X₁₀₅ and X₁₁₃ respectively. Without being bound by theory, the cysteines are preferably spaced so as to allow for the formation of a loop structure. X₁₀₄ and X₁₁₄ are both small amino acids such as, for example, alanine and glycine. Most preferably, X₁₀₄ is alanine and X₁₁₄ is glycine. X₁₀₅ may be any amino acid but is preferably valine. X₁₁₂ is preferably asparagine. Thus, the most preferred sequence is ACVWPTYWNCG. The IR binding displayed amino acid sequences are described in Figure 8.

- 9. An amino acid sequence comprising
 DYKDLCQSWGVRIGWLAGLCPKK (Formula 9, JBA5). The Formula 9
 motif is another motif believed to form a cysteine loop which possesses
 agonist activity at both IR and IGF-1R. Although IR binding is not detectable
 by ELISA, binding of Formula 9 to IR is competed by insulin and is agonistic.
 See Figure 11A. Binding of Formula 9 through IGF-1R is detected by
 ELISA. Nonlimiting examples of Formula 9 amino acid sequences are
 described in Figures 9A-9C.
- 10. WX_{123} GYX_{124} WX_{125} X_{126} (Formula 10, Group 6 Secondary Library) wherein X_{123} is selected from proline, glycine, serine, arginine, alanine or leucine, but more preferably proline; X_{124} is any amino acid, but preferably a charged or aromatic amino acid; X_{125} is a hydrophobic amino acid preferably leucine or phenylalanine, and most preferably leucine. X_{126} is any amino acid, but preferably a small amino acid. Nonlimiting examples of Formula 10 amino acid sequences are described in Figures 10A-10B.

11. Other Motifs

Another motif for use with this invention includes WPGY. Examples of specific peptide sequences comprising this motif include KVRGFQGGTVWPGYEWLRNAAKK (E8), and

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KSMFVAGSDRWPGYGVLADWLKK (F2).

Various amino acid sequences which bind IR and/or IGF-1R have been identified through panning of various libraries designed to identify preferred IR or IGF-1R consensus sequences which do not correspond to one of the motifs described above. Such sequences are described in Figures 10C-10I.

B. Amino And Carboxyl Terminal Extensions Modulate Activity of Motifs

In addition to the motifs stated above, the invention also provides preferred sequences at the amino terminal or carboxyl terminal ends which are capable of enhancing binding of the motifs to either IR, IGF-1R, or both. In addition, the use of the extensions described below does not preclude the possible use of the motifs with other substitutions, additions or deletions which allow for binding to IR, IGF-1R or both.

1. Formula 1

Any amino acid sequence may be used for extensions of the amino terminal end of A6, although certain amino acids in amino terminal extensions may be identified which modulate activity. Preferred carboxy terminal extensions for A6 are A6 X_{93} X_{94} X_{95} X_{96} X_{97} wherein X_{93} may be any amino acid, but is preferably selected from the group consisting of alanine, valine, aspartic acid, glutamic acid, and arginine, and X_{94} and X_{97} are any amino acid; X_{95} is preferably glutamine, glutamic acid, alanine or lysine but most preferably glutamine. The presence of glutamic acid at X_{95} however may confer some IR selectivity. Further, the failure to obtain sequences having an asparagine or aspartic acid at position X_{95} may indicate that these amino acids should be avoided to maintain or enhance sufficient binding to IR and IGF-1R. X_{96} is preferably a hydrophobic or aliphatic amino acid, more preferably leucine, isoleucine, valine, or tryptophan but most preferably leucine. Hydrophobic residues, especially tryptophan at X_{96} may be used to enhance IR selectivity.

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2. Formula 2

B6 with amino terminal and carboxy terminal extensions may be represented as X_{98} X_{99} B6 X_{100} . X_{98} is optionally aspartic acid and X_{99} is independently an amino acid selected from the group consisting of glycine, glutamine, and proline. The presence of an aspartic acid at X_{98} and a proline at X_{99} is associated with an enhancement of binding for both IR and IGF-1R. A hydrophobic amino acid is preferred for the amino acid at X_{100} , an aliphatic amino acid is more preferred. Most preferably leucine, for IR and valine for IGF-1R. Negatively charged amino acids are preferred at both the amino and carboxy terminals of Formula 2A.

3. Formula 3

An amino terminal extension of Formula 3 defined as X_{101} X_{102} X_{103} revB6 wherein X_{103} is a hydrophobic amino acid, preferably leucine, isoleucine or valine, and X_{102} and X_{101} are preferably polar amino acids, more preferably aspartic acid or glutamic acid may be useful for enhancing binding to IR and IGF-1R. No preference is apparent for the amino acids at the carboxy terminal end of Formula 3.

C. Secondary Structure

Without being bound by theory, it is believed that the B6 and reverse B6 motifs participate in alpha helix formation such that the most highly preferred residues at positions X_6 , X_7 , X_{10} and X_{13} (B6) and X_{14} X_{17} X_{20} and X_{21} (rB6) reside on the same side of a helix. See Figure 12. Because both B6 and RB6 motifs form structurally analogous motifs from their palindrome sequences, the use of D-amino acids instead of typical L-amino acids would be expected to produce amino acid sequences having similar properties to the L-amino acid sequences. D-amino acids may be advantageous, as the resultant sequences may be more resistant to enzymatic degradation than L-amino acid sequences. In addition, to maintain the appropriate orientation of highly preferred amino acid sequences on the appropriate side of the

helix, it is important to maintain the spacing of those residues along the amino acid sequence. For example, the second and third amino acids of B6 $(X_7 \text{ and } X_8)$ are oriented at opposite sides of the helix. See Figure 12.

D. IR Binding Preferences

As indicated above, the amino acid sequences containing the motifs of this invention may be constructed to have enhanced selectivity for either IR or IGF-1R by choosing appropriate amino acids at specific positions of the motifs or the regions flanking them. By providing amino acid preferences for IR or IGF-1R, this invention provides the means for constructing amino acid sequences with minimized activity at the non-cognate receptor. For example, the amino acid sequences disclosed herein with high affinity and activity for IR and low affinity and activity for IGF-1R are desirable as IR agonist as their propensity to promote undesirable cell proliferation, an activity of IGF-1 agonists, is reduced. Ratios of IR binding affinity to IGF-1R binding affinity for specific sequences are provided in Figures 1A-10I. As an insulin therapeutic, the IR/IGF-1R binding affinity ratio is preferably greater than 100. Conversely, for use as an IGF-1R therapeutic, the IR/IGF-1R ratio should be less than 0.01. Examples of peptides that selectively bind to IGF-1R are shown below.

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IGF-1R-SELECTIVE SEQUENCES

MOTIF 1 (A6-like):

	0	atios ove	Patios over Background		Comparisons	us
	. u	rance ere F-Tan	IGF-1R	쪼	IGF-1R/IR	IR/IGF-1R
Clone		કું - કું -	31 0	1.8	17.0	0.1
A6L-0-E6-IR	YRGMLVLGRSSDGAGKVAFERPARIGQTVFAVNFIDWFV 3	ς ν - α	ر ب د د د	9.0	16.0	0.1
H2CA-4-G9-IGFR	GIISQSCPESFYDWFAGQVSDPWWCW	٠		0.7	14.6	0.1
H2CA-4-H6-IGFR	VGRASGFPENFYDWFGRQLSLQSGEQ		16.0	1.3	13.0	0.1
A6L-0-E4-IR	YRGMLVLGRISDGAG#VASEPPARIGKKVFAVNFIDMIV 2	0.0		2.0	13.0	0.1
A6L-0-H3-IR	YRGMLVLGRISGGAGKAASERFARIGQRVSAVINFIDWIV Z		9.7	8.0	12.3	0.1
H2CA-4-F5-IGFR	VGYQGQGDENFYDWFIRQVSGKLGVQ	ה ה	. 6.	1.0	9.4	0.1
H2CA-4-H8-IGFR	SACQFDCHENFYDWFARQVSGGAAYG	•	, v	1.0	6.7	0.1
H2CA-4-F11-IGFR	SAAQLFFQESFYDWFLRQVAESSQPN	•		1.1	6.4	0.2
H2CA-4-F6-IGFR	AVRATRFDEAFYDWFVRQISUGQGNK	•	7.7	1.0	5.9	0.2
H2CA-4-F10-IGFR	VNQSGSIHENFYDWFERQVSHQRGVK	•	•	0.8	5.1	0.2
H2CA-1-A3-IGFR	APDPSDFQEIFYDWFVRQVSKMPGGG	•		1.2	4.8	•
H2CA-3-C8-IGFR	SSCDGAGHESFYEWFVRQVSGCRSV			1.7	4.2	0.2
H2CA-2-B9-IGFR	RAGSSDFHEDFYEWFVRQVSLSLKGK	•		1.0	4.2	0.2
H2CA-4-H4-IGFR	RQVS'I'GVGGG	} [6 6 6	8.5	4.1	0.2
$E4D\alpha-4-H2-IR$	•	•	•		4.1	0.2
H2CA-4-F7-IGFR	SSIGGEHENFYDWFSRQLSQSPPLK	٦ · ·	•		4.0	0.3
H2CA-3-D6-IGFR	QSPVGSSHEDFYDWFFRQVAQSGAHQ) o	•	•	4.0	0.3
H2CA-3-D8-IGFR	NYRRQVFNGNFYDWFDRQVFSLVI'PG	•	. o	2.5	3.9	0.3
H2CA-4-G11-IGFR	TLDGGSFEEQFYDWFVRQLSYRTNPD	•	. m	0.0	3.8	0.3
H2CA-4-F1-IGFR	FYVQQWGHENFYDWFDRQVSQSGGAG) . 		0.8	3.7	0.3
H2CA-3-D7-IGFR	LRRQAPVEENFYDWFVRQVSGDRVGG	n - a	2.0	0.6	3.7	0.3
H2CA-1-A7-IGFR	RCGRELYHSTFYDWFDRQVAGRICPS		4 1 T	7.	3.6	0.3
H2CA-2-B4-IGFR H2CA-2-B3-IGFR	CCLLCRFQQNFYDWFVCQGISKLKFL PPLASDLDVQFYGWFVQQVSPPGRGG	7.7	3.8	1.0	3.6	0.3

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		puno	Comparisons	18/1GF-1R
		F.Tag GF-1R K		
Clone	Sequence		3.5 0.3	
H2CA-2-B2-IGFR	GAPVDQLHEDFIDWFVRQVRAFIG	•	3.4 0.3	
$E4D\alpha-2-D11-IR$	GFREGSFYDWFQAQVT		3.3 0.3	
20E2Bβ-4-G6-IR	SQAGSAFYAWFDQVLRTVHSA	N C		
H2CA-4-H9-IGFR	RGAVAGFHDQFYDWFDRQVSKVHKFG) v	0.	
H2CA-2-B11-IGFR	AICDAGFHEHFYDWFALQVSDCGRQS) r	0	
H2CA-3-E8-IGFR	LGYQEPFQQNFYDWFVRQVSGAENAG	37 4	80	
A6S-2-D11-IR	EAASLGSQDRNFYDWFVRQVV	. · · · · · · · · · · · · · · · · · · ·	2.6 0.4	
A6S-2-D1-IR	VERSASSQDGNFYDWFVVQ1R	0 100	2.5 0.4	
A6S-3-E2-IR	TSEVQRRSQDNFYDWFVAQVA	7.42		
HOCA - 3 - FI11 - TGFR	HLADGQFHEKFYDWFERQISSRCNDC	4.7 2.2 I.O		
H2CA-3-C11-IGFR	FRTLAAQHDSFYDWFDRQVSGAAGER	9.3 3.3 L.6	>	
A6-PD1-IGFR	SFHEDFYDWFDRQVSGSLKK			
H2C-PD1-IGFR(RP9)	GSLDESFYDWFERQLGKK			

MOTIF 2 (B6-like):

		Ratios over	er Background	þ	Comparisons	ns
Clone	Sequence	E-Tag	IGF-1R	쪼	IGF-1R/IR	IR/IGF-1R
20C-3-G3-IGFR	TFYSCLASLLTGTPQPNRGPWERCR	33.1	32.3	1.2	27.0	<0.1
20C-4-C7-IGFR	FFYDCLAALLQGVARYHDLCAVEIT	35.3	28.0	1.3	21.8	<0.1
B6Hα-1-B5-IR	CCTTEMVVMDARDDPFYHKLSELVTGG	41.5	20.5	1.0	20.5	0.0
R20B-4-A6-IR	RGQSDAFYSGLWALIGLSDG	9.3	25.9	1.5	17.3	0.1
20E2B-1-A6-IGFR	GVRAMSFYDALVSVLGLGPSG	18.6	18.1	1.1	16.8	0.1
R20α-4-20A12-IR	RLFYCGIQALGANLGYSGCV	48.6	39.9	2.4	16.6	0.1
$20E2B\beta-4-G7-IR$	LQPCSGFYECIERLIGVKLSG	19.9	25.2	1.6	15.8	0.1
NNRPy-4-B11-IR	LKDGFYDYFWQRLHLGS	4.1	18.7	1.2	15.5	0.1
20E2B-3-C6-IGFR	VEGRGLFYDLLRQLLARRQNG	17.9	16.8	1.	14.8	0.1
B6Hα-1-A2-IR	RGCNDDGGKGWSDDPFYHKLSELICGG	22.3	14.6	1.0	14.6	0.1
20E2A-4-F11-IGFR	QGGSASFYDAIDRLLRMRIGG	21.3	18.8	1.3	14.6	0.1
B6Hα-3-E9-IR	RCEEKQAEVGPSSDPFYHKMSELLGCR	44.6	24.2	1.7	14.2	0.1
20C-3-F6-IGFR	DRDFCRFYERLTALVGGQVDGGWPC	33.5	26.1	1.9	14.1	0.1
20E2B-4-H3~IGFR	KLHNLMFYYGLQRLVWGAGLG	11.2	14.8	1.1	13.9	0.1
20E2B-3-C2-IGFR	GNGDGMFYQLLSLLVGRDMHV	13.1	8	9.0	13.8	0.1
20C-3-A1-IGFR	SSYGCDGFYLMLFSLGLVASQELEC	26.5	20.8	1.5	13.7	0.1
20E2B-3-E3-IGFR	PDLHKGFYAQLAQLIRGQLLS	22.4	16.3	1.3	13.1	0.1
R200-3-20E2-IR	FYDAIDQLVRGSARAGGTRD	46.3	39.9	3.1	12.9	0.1
20E2B-4-H12-IGFR	YSCGDGFYSLLSDLLGGQFRC	6.5	6.7	0.8	12.8	0.1
$B6H\alpha-3-F11-IR$	RGMKEEVLVGGSTDPFYHKLSELLQGS	49.5	18.7	1.6	11.7	0.1
20E2B-3-D2-IGFR	IQQELTFYDLLHRLVRSELGS	20.7	12.4	1.1	11.7	0.1
20E2B-3-D8-IGFR	GGTEVDFYRALERLVRGQLGL	20.4	17.7	1.6	11.3	0.1
20E2B-3-E8-IGFR	LRIANLFYQRLWDLAFGGGG	15.7	16.7	1.5	11.1	0.1
B6Hα-2-C4-IR	RCGRW*AEMGAGDDPFYHKLSELVCG	20.7	0.0	6.0	11.0	0.1
R20a-4-20C11-IR	DRAFYNGLRDLVGAVYGAWD	43.7	30.8	3.0	10.3	0.1
20E2B-4-F8-IGFR	PVGVQGFYEGLSRLVLGRGGW	12.3	7.3	0.8	9.7	0.1

		Ratios ove	over Background	þ	Comparisons	suc
		F-Tan	IGF-1R	坚	IGF-1R/IR	IR/IGF-1R
Clone		і - п В С	, LJ.	1.0	9.7	0.1
20E2B-1-A11-IGFR	RFSTDGFYQYDDADVGGGFVG) c	•	8	9.6	0.1
20E2B-3-D4-IGFR		o (. <	•	4.	0.1
20E2B-2-B11-IGFR				+ α •	σ	0.1
20E2B-3-C8-IGFR	QPAPDGFYSALMKLIGRGGVS	T8.5	n			-
10円2 20日2日-2-B2-1GFR	PGTDLGFYQALRCVVIQGACD	11.7	4.9	9.0	- · ·	+ + •
20世紀10 2 世紀 10年17. 20年2日 7 - 再10 - 172年18	AOPCGGFYGI,LEOLVGRSVCD	19.0	17.3	•	φ	T .
ZOEZB-4-FIO-IGFIC		11.9	14.7	1.9	7.7	T.0
20E2B-4-F9-1GFR			8.8	1.2	7.6	0.1
20C-3-A4-1GFR		14.3	12.2	1.6	7.6	0.1
20E2B-3-D11-IGFR		•	15.4	•	7.5	0.1
20E2B-3-C11-IGFR	CMM. DGFYAGLGCLLTAGEGR	•	л н . ч	7.0	7.4	0.1
20E2B-2-B3-IGFR	ICTGQGFYQVLCGLLRGTSAK	, ,	•	•	7.3	0.1
20E2B-3-D12-IGFR	QGNVLDFYGWIGRLLAKQGSD	٠	•	•	7 . 7	
20E2B-3-E12-IGFR	VATSQGFYSGLSELLQGGGNV	13.9	0.9	٠	•	H
200202-2-B8-TGFR	IWATGDFYRLLSQLVMGRVGT	17.4	5.7	æ. O	•	· · ·
	50.15.1.1244V2V4C0C4	3.0	10.0	1.4	7.1	1.0
$NNRF\gamma - 4 - A9 - 1R$			7	9.0	7.0	0.1
20E2B-4-G11-IGFR	ROGTGSFYLIMLEQULVGARGE	• (. [6.9	0.1
20E2B-3-D6-IGFR	DSVGDNFYQLLESLVGGHGVG	N	-	•		C
BEHW-0-C7-TR	RGIVAMVEATEVGSDHDPFYHKLSELVQGS	GS45.1	6.7	•	٠	
DOMEST () III	T. C.	18.0	6.1	o.0	٠	T. (
ZUEZE-Z-BZ-ZOEZ		23.4	20.4	3.3	6.2	7.0
20E2B-3-C4-1GFK			7.7	1.3	6.0	0.2
20C-3-E4-IGFR				~	0.9	0.2
NNRPy-4-A1-IR	IIGGFYSYFNSVLRLGT	•)) C	o o	0.2
20E2B-4-H8-IGFR	PAGPCGFYCGLGLLLHGDQSP	•	ກ ເ	•	•	· · ·
20E2B-4-H9-IGFR	RCQGTGFYTCIQELIGFGDPD	4.5	5.2). V	•	1 (
	SDOTTENAMEDSCHOLLOGS	46.9	5.8	H.	5.3	7.0
BOHQ-Z-CIO-IR		17.6	5.4	⊣.	5.1	7.0
20E2A-3-C/-1GFR		16.1	4.4	6.0	5.0	0.2
20E2B-1-A8-1GFK			رب ب	ر . در	4.9	0.2
NNRPy-4-A7-IR	RFDPFYSYFVNLLGASA	ر. ن)) •		

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	- 4+ -					
	2	atios ove	Ratios over Background	70	Comparisons	ns
i		E-Tag	IGF-1R	豆	IGF-1R/IR	K/ GF-17
Clone)) 	y (٦٦	←	4.8	0.2
B6Hα-3-E8-IR	н .	•	•	0	7	0.2
7-014-3-8H90	GCVVEWOKWHGASDPFYHKLSELGGCS	7.7	α.	· -	•	
	3 STATES OF THE SELVICE STATES OF THE SELVIC	33.5	4.4	1.0	4.4	7.0
В6Нα-2-П6-1К	ACTION TO THE THIRT TO US OF THE TOTAL TO US OF THE TAIL TO US OF THE TOTAL TO US OF THE	0 41	5.6	1.3	4.3	0.2
$B6H\alpha - 3 - E7 - IR$	GCAVVEEAERSRGDFFTHRLDELLQGC	· ·	Д	1.3	4.2	0.2
$B6H\alpha-2-D1-IR$	GCEVIVEEGDSADPFYHKLSELCQGS	· · · · ·		1.8	3.9	0.3
20E2A-3-D10-IGFR	N 1		•		3.9	0.3
20E2A-3-A12-IGFR	-1 C)	7 1	3.6	0.3
B6Hα-4-G8-IR	GGTKAVAKVGTRDDPFYHKLSELLQGS	0.7		4	3.4	0.3
B6L-4-D7-IR	AETSVQVGWIRLQSVWPGEHWNTVDPFYHKLSELLKGSGA14	•	•	•		۲,
BEHG-1-A3-TR	SRAKVEAEMPDSGDPFYHKLSELLASG	37.4	7.0	о Э) (
	4	41.5	3.1	1.0	3. F	0.0
B6HQ-3-F1-1K		19.3	3.0	1.0	3.0	0.3
B6Hα-2-D8-IR	2	1 66	6	←! -!	2.9	0.3
$B6H\alpha-1-B3-IR$				C	2.0	0.3
B6Hα-3-E5-IR	SELFGGC			· ·		0.4
20F2A-4-G11-IGFR	MNVFVSFYDAIDQLVCQRIGC	7.07	0.0) l	•	С Ц
01 LD C 840400		1.5	3.1	٦.5	0.7	•
ZOEZBD-3-(OEZBDZ		18.2	2.3	1.2	1.9	ი.ა
B6Hα-3-E6-1R		2.2	3.0	1.5	1.9	o.5
20E2A-3-A3-IGFR	GHYFGSFYDALDQLvAcmined	,	3.4	1.9	1.8	9.0
B6L-4-A7-IR	AGT PAQVG*NKLWSVWFGBINNI VDF I INITIOLIFICE COM-		2.4	1.3	1.8	0.5
$B6H\alpha - 3 - F1 - IR$	CSMAAVAEAGDDDDFYHKLSELCQGS	1 • (1.8	1.4	0.7
B6L-3-G6-IR	VDTPAQVGWNRLWSVGFGEHWILDDFFIR. LISELLINGSOM.	• 1	2.0	1.4	1.4	0.7
B6L-3-G5-IR	AETSAQVGWQKLWSVWFGDRWSILDFFIIINLSHLLLING 2 COTTENDAMBOI WATCHGA		2.5	1.8	1.4	0.7
20E2A-3-A4-IGFR	AGSVISFIDAMEQUVAIGISA					

TDDGFYDALEQLVQGSKK GSFYEALQRLVGGEQGKK B6-PD1-IGFR 20E2-PD1-IGFR (RP10)

MOTIF 10 (Group6):

		Ratios ove	Ratios over Background	ס	Comparisons	SL
Ī		E-Tag	IGF-1R	≅	IGF-1R/IR	IR/IGF-1R
Clone	acinanhac	, ,	0 70	رب د	7.6	0.1
R208-4-E8-IR	VRGFQGGTVWPGYEWLRNAA	4. L) - -			,
10F-4-D1-TGFR	1,SCT,AYSRHGIWRPSTDLGLGRSVGEGSVSTRWRGYDWFE	4.9	4.6	າ.	7.7	· ·
101 T TOT	LECTION OF THE PROPERTY OF THE	4.1	3.0	0.5	13.1	٦.٠
40F-4-B1-1GFR	GLUHSUAVGVHLGFAWFAQARGRAMADGGGATATA	l (L4	4	11.7	0.1
40F-4-D10-IGFR	W.GYAWLS	4. Y	1 ,	ተ • ጋ	• , 	
R20β-4-E8-IR	VRGFQGGTVWPGYEWLRNAA	41.0	3.6	34.9	0.1	

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Besides relative binding at IR or IGF-1R, relative efficacy at the cognate receptor is another important consideration for choosing a potential therapeutic. Thus, a sequence which is efficacious at IR but has little or no significant activity at IGF-1R may also be considered as an important IR therapeutic, irrespective of the relative binding affinities at IR and IGF-1R.

A6 selectivity for IR may be enhanced by including glutamic acid in a carboxyl terminal extension at position X_{95} . IR selectivity of the B6 motif may be enhanced by having a tryptophan or phenylalanine at X_{11} . Tryptophan at X_{13} also favors selectivity of IR. A tryptophan amino acid at X_{13} rather than leucine at that position also may be used to enhance selectivity for IR. In the reverse B6 motif, a large amino acid at X_{15} favors IR selectivity. Conversely, small amino acids may confer specificity for IGF-1R. In the F8 motif, an L in position X_{23} is essentially required for IR binding. In addition, tryptophan at X_{31} is also highly preferred. At X_{32} , glycine is preferred for IR selectivity.

E. Multiple Binding Sites On IR And IGF-1R

The competition data disclosed herein reveals that at least two separate binding sites are present on IR and IGF-1R which recognize the different sequence motifs provided by this invention.

As shown in Figure 13, competition data (See Example 15) indicates that peptides comprising the A6, B6, revB6, and F2 motifs compete for binding to the same site on IR (Site 1) whereas the F8 and D8 motifs compete for a second site (Site 2). Similarly, the decrease of dissociation of B6 motif peptide (20E2) from IGF-1R by a D8 ligand indicates multiple interacting binding sites.

The identification of peptides which bind to separate binding sites on IR and IGF-1R provides for various schemes of binding to IR or IGF-1R to increase or decrease its activity. Examples of such schemes for IR are illustrated in Figure 15.

The table below shows sequences based on their groups, which bind to Site 1 or Site 2.

REPRESENTATIVE SITE 1 PEPTIDES

A6-like	(FYxWF):
---------	----------

	Clone	Sequence
5	G3	KRGGGTFYEWFESALRKHGAGKK
_	Н2	VTFTSAVFHENFYDWFVRQVSKK
	H2C	FHENFYDWFVRQVSKK
	A6S-IR3-E12	GRVDWLQRNANFYDWFVAELG
	A6S-IR4-G1	NGVERAGTGDNFYDWFVAQLH
10	H2CB-R3-B12	QSDSGTVHDRFYGWFRDTWAS
	20E2A-R3-B11	GRFYGWFQDAIDQLMPWGFDP
	rB6-F6	RYGRWGLAQQFYDWFDR
	E4Dα-1-B8-IR~	GFREGQRWYWFVAQVT
15	H2CA-4-F11-IR	TYKARFLHENFYDWFNRQVSQYFGRV
	H2CB-R3-D2	WTDVDGFHSGFYRWFQNQWER
	H2CB-R3-D12	VASGHVLHGQFYRWFVDQFAL
	H2CB-R4-H5	QARVGNVHQQFYEWFREVMQG
	H2C-B-E8*	TGHRLGLDEQFYWWFRDALSG
20	H2CB-3-B6-IR~	VGDFCVSHDCFYGWFLRESMQ
	A6S-IR2-C1	RMYFSTGAPQNFYDWFVQEWD

B6-like (FYxxLxxL): Clone

	50 mile (: 1707—70	
	Clone	Sequence
25	20C11	KDRAFYNGLRDLVGAVYGAWDKK
	20E2	DYKDFYDAIDQLVRGSARAGGTRDKK
	B62-R3-C7	EHWNTVDPFYFTLFEWLRESG
	B62-R3-C10	EHWNTVDPFYQYFSELLRESG
30	20E2B-3-B3-IR	AGVNAGFYRYFSTLLDWWDQG
	20E2-B-E3*	IQGWEPFYGWFDDVVAQMFEE
	20E2A-R4-F9	PPWGARFYDAIEQLVFDNLCC
	RPNN-4-G6-HOLO*	RWPNFYGYFESLLTHFS
	RPNN-4-F3-HOLO*	HYNAFYEYFQVLLAETW
35	20E2A-R4-E2	IGRVRSFYDAIDKLFQSDWER
	RPNN-2-C1-IR*	EGWDFYSYFSGLLASVT
	20E2B-4-F12-IR	SVKEVQFYRYFYDLLQSEESG
	20E2-B-E12	GNSGGSFYRYFQLLLDSDGMS
	20E2A-R3-B6	RDAGSSFYDAIDQLVCLTYFC
40		

Reverse B6-like (LxxLxxYF): Clone Sequence

CIONE	56946116
rB6-A12	LDALDRLMRYFEERPSL
rB6-F9	PLAELWAYFEHSEQGRSSAH
rB6-4-E7-IR	LDPLDALLQYFWSVPGH
	-
rB6-4-F9-IR	RGRLGSLSTQFYNWFAE
rB6-E6	ADELEWLLDYFMHQPRP
rB6-4-F12-IR	DGVLEELFSYFSATVGP

Group 6 (WPxYxWL):

O. 0 a p o (
Clone	Sequence
R20B-4-A4-TR	WPGYLFFEEALQDWRGSTED

55 Peptides by design**:

, ,	i epilacs by acoig	•	
	Clone	Sequence	
	H2C-PD1-IR~	AAVHEQFYDWFADQYKK	
	A6S-PD1-IR~	QAPSNFYDWFVREWDKK	
	20E2-PD1-IR~	OSFYDYIEELLGGEWKK	
08	B6C-DD1-TR~	DPFYOGLWEWLRESGKK	

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REPRESENTATIVE SITE 2 PEPTIDES (C-C LOOPS)

F8-derived (Long C-C loop):

	Clone	Sequence			
5	F8	HLCVLEELFWGASLFGYCSG			
	F8-C12	FQSLLEELVWGAPLFRYGTG			
	F8-Des2	PLCVLEELFWGASLFGYCSG			
10	F8-F12 F8-B9 F8-B12	PLCVLEELFWGASLFGQCSG HLCVLEELFWGASLFGQCSG DLRVLCELFGGAYVLGYCSE			
15	NNKH-2B3 NNKH-2F9~ NNKH-4H4~	HRSVLKQLSWGASLFGQWAG HLSVGEELSWWVALLGQWAR APVSTEELRWGALLFGQWAG			

D8-derived (Small C-C loop):

Clone	Sequence
D8	KWLDQEWAWVQCEVYGRGCPSKK
D8-G1	QLEEEWAGVQCEVYGRECPS
D8-B5~	ALEEEWAWVQVRSIRSGLPL
D8-A7	SLDQEWAWVQCEVYGRGCLS
D8-F1~	WLEHEWAOIOCELYGRGCTY

	Clone	Sequence
	D8-F10	GLEQGCPWVGLEVQCRGCPS
	F8-B12~	DLRVLCELFGGAYVLGYCSE
30	F8-A9	PLWGLCELFGGASLFGYCSS

^{**}Based on analysis of entire panning data, amino acid preferences at each position were calculated to define these "idealized" peptides.

- 35 * Peptides synthesized and currently being purified
 - ~ Peptides planned

F. **Multivalent Ligands**

This invention provides ligands which preferentially bind different 40 sites on IR and IGF-1R. The amino acid motifs which bind IR at one site (Site 1, Figure 13) are A6, B6, revB6, and F2. A second in site (Site 2, Figure 13) binds F8 and D8. Accordingly, multimeric ligands may be prepared according to the invention by covalently linking amino acid sequences. Depending on the purpose intended for the multivalent ligand, 45 amino acid sequences which bind the same or different sites may be combined to form a single molecule. Where the multivalent ligand is constructed to bind to the same corresponding site on different receptors, or

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different subunits of a receptor, the amino acid sequences of the ligand for binding to the receptors may be the same or different, provided that if different amino acid sequences are used, they both bind to the same site.

Multivalent ligands may be prepared by either expressing amino acid sequences which bind to the individual sites separately and then covalently linking them together, or by expressing the multivalent ligand as a single amino acid sequence which comprises within it the combination of specific amino acid sequences for binding.

Various combinations of amino acid sequences may be combined to produce multivalent ligands having specific desirable properties. Thus, agonists may be combined with agonists, antagonists combined with antagonists combined with antagonists. Combining amino acid sequences which bind to the same site to form a multivalent ligand may be useful to produce molecules which are capable of cross-linking together multiple receptor units. Multivalent ligands may also be constructed to combine amino acid sequences which bind to different sites (Figure 15).

In view of the discovery disclosed herein of monomers having agonist properties at IR or IGF-1R, preparation of multivalent ligands may be useful to prepare ligands having more desirable pharmacokinetic properties due to the presence of multiple bind sites on a single molecule. In addition, combining amino acid sequences which bind to different sites with different affinities provides a means for modulating the overall potency and affinity of the ligand for IR or IGF-1R.

1. Construction of Hybrids

In one embodiment, hybrids of at least two peptides may be produced as recombinant fusion polypeptides which are expressed in any suitable expression system. The polypeptides may bind the receptor as either fusion constructs containing amino acid sequences besides the ligand binding sequences or as cleaved proteins from which signal sequences or other sequences unrelated to ligand binding are removed. Sequences for facilitating purification of the fusion protein may also be expressed as part of

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the construct. Such sequences optionally may be subsequently removed to produce the mature binding ligand. Recombinant expression also provides means for producing large quantities of ligand. In addition, recombinant expression may be used to express different combinations of amino acid sequences and to vary the orientation of their combination, i.e., amino to carboxyl terminal orientation.

Whether produced by recombinant gene expression or by conventional chemical linkage technology, the various amino acid sequences may be coupled through linkers of various lengths. Where linked sequences are expressed recombinantly, and based on an average amino acid length of about 4 angstroms, the linkers for connecting the two amino acid sequences would typically range from about 3 to about 12 amino acids corresponding to from about 12 to about 48 Å. Accordingly, the preferred distance between binding sequences is from about 2 to about 50 Å. More preferred is 4 to about 40. The degree of flexibility of the linker between the amino acid sequences may be modulated by the choice of amino acids used to construct the linker. The combination of glycine and serine is useful for producing a flexible, relatively unrestrictive linker. A more rigid linker may be constructed by using amino acids with more complex side chains within the linkage sequence.

In a preferred embodiment shown below (Figure 16)

MBP-FLAG-PEPTIDE-(G,S)n-PEPTIDE-E-TAG

a fusion construct producing a dipeptide comprises a maltose binding protein amino acid sequence (MBP) or similar sequence useful for enabling the affinity chromatography purification of the expressed peptide sequences. This purification facilitating sequence may then be attached to a flag sequence to provide a cleavage site to remove the initial sequence. The peptide dimer then follows which includes the intervening linker and a tag sequence may be included at the carboxyl terminal portion to facilitate identification/purification of the expression of peptide. In the representative

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construct illustrated above, G and S are glycine and serine residues, which make up the linker sequence.

In addition to producing the dimer peptides by recombinant protein expression, dimers may also be produced by peptide synthesis whereby a synthetic technique such as Merrifield synthesis (Merrifield, 1997), may be used to construct the entire peptide.

Other methods of constructing dimers include introducing a linker molecule which activates the terminal end of a peptide so that it can covalently bind to a second peptide. Examples of such linkers include diaminoproprionic acid activated with an oxyamino function. A preferred linker is a dialdehyde having the formula

$$O=CH-(CH_2)_n-CH=O$$
,

Wherein n is 2 to 6, but is preferably 6 to produce a linker of about 25 to 30 angstroms in length. Linkers may be used to link dimers either to the carboxyl terminal or the amino terminal.

2. Characterization Of Specific Dimers

Specific dimers which bind with high affinity to Site 1, Site 2, or both Site 1 and Site 2 of the insulin receptor are shown in Table 1. Although agonist activity has been observed for the Site 1-Site 1 dimers, the Site 1-Site 2 dimers may also possess desirable properties.

TABLE 1

Fusion	Seq.	Action	Site	Fusion Concentration	MW (kDa)	K _d (HIR)
426	D8	N	2	0.76	52.2	1.4 x 10 ⁻⁶
429	D8-6aa-D8	N-N	2-2	3.2	55.3	1.3 x 10 ⁻⁶
430	H2C-6aa-RB6	A-	1-1	0.17	54.5	2.1 x 10 ⁻⁶
431	H2C-6aa-F8	A-N	1-2	3.3	54.8	4.7 × 10 ⁻⁸
432	H2C-12aa-F8	A-N	1-2	2.9	55.5	3.5 x 10 ⁻⁸
433	H2C-9aa-F8	A-N	1-2	2.8	55.2	2.1 x 10 ⁻⁸
434	G3-12aa-G3	N-N	1-1	0.01	56	3.2 x 10 ⁻⁶
436	H2C-9aa-H2C	Α	1-1	1.1	54.2	4.1 × 10 ⁻⁷
437	H2C	N-N	1	0.3	51.5	8.3 x 10 ⁻⁶
427	G3-6aa-G3	N-N	1-1	0.02	55.3	3.3 x 10 ⁻⁶
435	H2C-3-H2C-3-H2C	A-A-A	1-1-1	2.1	55.5	2.0 x 10 ⁻⁶
439	H2C-6aa-H2C	A-A	1-1	1.4	53.9	5.5 x 10 ⁻⁷
449	H2C-12aa-H2C		1-1	1.5	51.8	6.2 x 10 ⁻⁷
452	G3		1	0.15	48.8	7.8 x 10 ⁻⁷
463	H2C-3aa-H2C	A-A	1-1	1.8	50.1	9.6 x 10 ⁻⁷
464	LF-H2C		1	0.045	48.4	3.9 x 10 ⁻⁸
446	LF-F8		2	1.9	49.1	7.7 x 10 ⁻⁷
459	SF-RB6			0.069	48.1	7.7 x 10 ⁻⁸
MBP*	lacZ			5.1	50	> 1 x 10 ⁻⁵

^{*}MBP (negative control for the fusions) is fused to a small fragment of beta-galactosidase (lacZ).

LF = Long FLAG epitope (DYKDDDDK)

Additional binding data for the fusion peptides are shown below:

Fusion	Highest conc. tested (μΜ)	Kd (HIR) μM
431-	0.2	0.033
431+	0.2	0.0074
432-	0.2	0.02
432+	0.2	0.0038
433-	0.2	0.03
433+	0.2	0.004

The concentrations of these fusions vary depending on the expression quality. There are 2 sets of each fusion: uncleaved (-) and cleaved with factor Xa (+). The fusion proteins are in Tris buffer (20 mM Tris, 200 mM NaCl, 1 mM EDTA, 50 mM maltose, pH 7.5) and the cleaved fusions (+) are in the same Tris buffer (500 μ l) + 12 μ g Factor Xa. (Source of Factor Xa: New England Biolabs).

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N = Antagonist

A = Agonist

SF = Short FLAG epitope (DYKD)

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Other combinations of peptides are within the scope of this invention and may be determined as demonstrated in the examples described herein.

Regarding preparation of a Site 1 agonist comprising two D117 (H2C) peptides, a linker of only 3 amino acids (12 Å) provided a ligand of greater affinity for Site 1 of IR than a corresponding ligand prepared with a 9 amino acid (36 Å) linking region. Figure 17.

Notably, several fusion peptides show IR agonist activity as determined by an IR autophosphorylation assay (see Example 20). Figure 74. In particular, fusion peptides 439, 436, 449, and 463 show significant IR agonist activity (Figure 74).

G. Peptide Synthetic Techniques

Many conventional techniques in molecular biology, protein biochemistry, and immunology may be used to produce the amino acid sequences for use with this invention.

1. <u>Recombinant Synthesis</u>

To obtain recombinant peptides, the corresponding DNA sequences may be cloned into any suitable vectors for expression in intact host cells or in cell-free translation systems by methods well known in the art (see Sambrook *et al.*, 1989). The particular choice of the vector, host, or translation system is not critical to the practice of the invention.

Cloning vectors for the expression of recombinant peptides include, but are not limited to, pUC, pBluescript (Stratagene, La Jolla, CA), pET (Novagen, Inc., Madison, WI), pMAL (New England Biolabs, Beverly, MA), or pREP (Invitrogen Corp., San Diego, CA) vectors. Vectors can contain one or more replication and inheritance systems for cloning or expression, one or more markers for selection in the host (e.g. antibiotic resistance), and one or more expression cassettes. The inserted coding sequences can be synthesized by standard methods, isolated from natural sources, or prepared as hybrids, etc. Ligation of the coding sequences to transcriptional regulatory elements and/or to other amino acid coding sequences can be

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carried out using established methods. DNA sequences can be optimized, if desired, for more efficient expression in a given host organism. For example, codons can be altered to conform to the preferred codon usage in a given host cell or cell-free translation system using techniques routinely practiced in the art.

Suitable cell-free systems for expressing recombinant peptides include, for example, rabbit reticulocyte lysate, wheat germ extract, canine pancreatic microsomal membranes, *Escherichia coli* (*E. coli*) S30 extract, and coupled transcription/translation systems (Promega Corp., Madison, WI). Such systems allow expression of recombinant polypeptides upon the addition of cloning vectors, DNA fragments, or RNA sequences containing coding regions and appropriate promoter elements.

Host cells for cloning vectors include bacterial, archebacterial, fungal, plant, insect and animal cells, especially mammalian cells. Of particular interest are *E. coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Neurospora crassa*, SF9, C129, 293, NIH 3T3, CHO, COS, and HeLa cells. These cells can be transformed, transfected, or transduced, as appropriate, by any suitable method including electroporation, CaCl₂-, LiCl-, LiAc/PEG-, spheroplasting-, Ca-Phosphate, DEAE-dextran, liposome-mediated DNA uptake, injection, microinjection, microprojectile bombardment, or other established methods.

For some purposes, it may be preferable to produce peptides in a recombinant system in which they carry additional sequence tags to facilitate purification. Non-limiting examples of tags include c-myc, haemagglutinin (HA), polyhistidine (6X-HIS), GLU-GLU, and DYKDDDDK (FLAG®) epitope tags. Epitope tags can be added to peptides by a number of established methods. DNA sequences of epitope tags can be inserted into peptide coding sequences as oligonucleotides or through primers used in PCR amplification. As an alternative, peptide coding sequences can be cloned into specific vectors that create fusions with epitope tags; for

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example, pRSET vectors (Invitrogen Corp., San Diego, CA). The expressed, tagged peptides can then be purified from a crude lysate of the cell-free translation system or host cell by chromatography on an appropriate solid-phase matrix.

Methods for directly purifying peptides from natural sources such as cellular or extracellular lysates are well known in the art (see Harris and Angal, 1989). Such methods include, without limitation, sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE), preparative disc-gel electrophoresis, isoelectric focusing, high-performance liquid chromatography (HPLC), reversed-phase HPLC, gel filtration, ion exchange and partition chromatography, countercurrent distribution, and combinations thereof. Naturally occurring peptides can be purified from many possible sources, for example, plasma, body tissues, or body fluid lysates derived from human or animal, including mammalian, bird, fish, and insect sources.

Antibody-based methods may also be used to purify naturally occurring or recombinantly produced peptides. Antibodies that recognize these peptides or fragments derived therefrom can be produced and isolated. The peptide can then be purified from a crude lysate by chromatography on an antibody-conjugated solid-phase matrix (see Harlow and Lane, 1998).

2. Chemical Synthesis Of Peptides

Alternately, peptides may be chemically synthesized by commercially available automated procedures, including, without limitation, exclusive solid phase synthesis, partial solid phase methods, fragment condensation or classical solution synthesis. The polypeptides are preferably prepared by solid-phase peptide synthesis; for example, as described by Merrifield (1965; 1997). In addition, recombinant and synthetic methods of polypeptide production can be combined to produce semi-synthetic polypeptides.

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H. Screening Assays

In another embodiment of this invention, screening assays to identify pharmacologically active ligands at IR and/or IGF-1R are provided. The screening assays provided in accordance with this invention are based on those disclosed in International application WO 96/04557 which is incorporated herein in its entirety. Briefly, WO 96/04557 discloses the use of reporter peptides which bind to active sites on targets and possess agonist or antagonist activity at the target. These reporters are identified from recombinant libraries and are either peptides with random amino acid sequences or variable antibody regions with at least one CDR region which has been randomized (rVab). The reporter peptides may be expressed in cell recombinant expression systems, such as for example in E. coli, or by phage display. See WO 96/04557 and Kay et al. (1996), both of which are incorporated herein by reference. The reporters identified from the libraries may then be used in accordance with this invention either as therapeutics themselves, or in competition binding assays to screen for other molecules, preferably small, active molecules, which possess similar properties to the reporters and may be developed as drug candidates to provide agonist or antagonist activity. Preferably, these small organic molecules are orally active.

The basic format of an *in vitro* competitive receptor binding assay as the basis of a heterogeneous screen for small organic molecular replacements for insulin may be as follows: occupation of the active site of IR is quantified by time-resolved fluorometric detection (TRFD) with streptavidin-labeled europium (saEu) complexed to biotinylated peptides (bP). In this assay, saEu forms a ternary complex with bP and IR (i.e., IR:bP:saEu complex). The TRFD assay format is well established, sensitive, and quantitative (Tompkins *et al.*, 1993). The assay can use a single-chain antibody or a biotinylated peptide. Furthermore, both assay formats faithfully report the competition of the biotinylated ligands binding to the active site of IR by insulin.

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In these assays, soluble IR is coated on the surface of microtiter wells, blocked by a solution of 0.5% BSA and 2% non-fat milk in PBS, and then incubated with biotinylated peptide or rVab. Unbound bP is then washed away and saEu is added to complex with receptor-bound bP. Upon addition of the acidic enhancement solution, the bound europium is released as free Eu³⁺ which rapidly forms a highly fluorescent and stable complex with components of the enhancement solution. The IR:bP bound saEu is then converted into its highly fluorescent state and detected by a detector such as Wallac Victor II (EG&G Wallac, Inc.)

The designing of mimetics to a known pharmaceutically active compound is a known approach to the development of pharmaceuticals based on a "lead" compound. This might be desirable where the active compound is difficult or expensive to synthesize or where it is unsuitable for a particular method of administration, e.g. peptides are generally unsuitable active agents for oral compositions as they tend to be quickly degraded by proteases in the alimentary canal. Mimetic design, synthesis and testing is generally used to avoid randomly screening large number of molecules for a target property.

There are several steps commonly taken in the design of a mimetic from a compound having a given target property. First, the particular parts of the compound that are critical and/or important in determining the target property are determined. In the case of a peptide, this can be done by systematically varying the amino acid residues in the peptide (e.g. by substituting each residue in turn). These parts or residues constituting the active region of the compound are known as its "pharmacophore".

Once the pharmacophore has been found, its structure is modeled according to its physical properties (e.g. stereochemistry, bonding, size and/or charge), using data from a range of sources (e.g. spectroscopic techniques, X-ray diffraction data and NMR). Computational analysis, similarity mapping (which models the charge and/or volume of a

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pharmacophore, rather than the bonding between atoms), and other techniques can be used in this modeling process.

In a variant of this approach, the three dimensional structure of the ligand and its binding partner are modeled. This can be especially useful where the ligand and/or binding partner change conformation on binding, allowing the model to take account of this in the design of the mimetic.

A template molecule is then selected onto which chemical groups which mimic the pharmacophore can be grafted. The template molecule and the chemical groups grafted on to it can conveniently be selected so that the mimetic is easy to synthesize, is likely to be pharmacologically acceptable, does not degrade *in vivo*, and retains the biological activity of the lead compound. The mimetics found are then screened to ascertain the extent they exhibit the target property, or to what extent they inhibit it. Further optimization or modification can then be carried out to arrive at one or more final mimetics for *in vivo* or clinical testing.

This invention provides specific IR and IGF-1R amino acid sequences which function as either agonists or antagonists at IR and/or IGF-1R. Examples of phage display libraries suitable for use in this invention include one such library containing randomized 40 amino acid peptides (RAPIDLIBTM, Figure 16), another library containing rVab derived from human genomic antibody DNA (GRABLIBTM, Figure 30). Details of the construction and analyses of these libraries, as well as the basic procedures for biopanning and selection of binders, have been described elsewhere (WO 96/04557; Mandecki *et al.*, 1997; Ravera *et al.*, 1998; Scott and Smith, 1990); Grihalde *et al.*, 1995; Chen *et al.*, 1996; Kay *et al.*, 1993, Carcamo *et al.*, 1998, all of which are incorporated herein by reference). Another phage display library suitable for use with this invention is available commercially from New England Biolabs (Ph.D. C7C Disulfide Constrained Peptide Library). Additional sequences may be obtained in accordance with the procedures described herein.

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plasmid) to a protein ligand via polylysine. The appropriate or suitable ligands are selected on the basis of the presence of the corresponding ligand receptors on the cell surface of the target cell or tissue type. These ligand-DNA conjugates can be injected directly into the blood, if desired, and are directed to the target tissue where receptor binding and DNA-protein complex internalization occur. Co-infection with adenovirus to disrupt endosome function can be used to overcome the problem of intracellular destruction of DNA.

An approach that combines biological and physical gene transfer methods utilizes plasmid DNA of any size combined with a polylysine-conjugated antibody specifically reactive with the adenovirus hexon protein. The resulting complex is bound to an adenovirus vector. The trimolecular complex is then used to infect cells. The adenovirus vector allows efficient binding to the cell, internalization, and degradation of the endosome before the coupled DNA can be damaged.

Many types of cells and cell lines (e.g. primary cell lines or established cell lines) and tissues are capable of being stably transfected by or receiving the constructs of the invention. Examples of cells that may be used include, but are not limited to, stem cells, B lymphocytes, T lymphocytes, macrophages, other white blood lymphocytes (e.g. myelocytes, macrophages, monocytes), immune system cells of different developmental stages, erythroid lineage cells, pancreatic cells, lung cells, muscle cells, liver cells, fat cells, neuronal cells, glial cells, other brain cells, transformed cells of various cell lineages corresponding to normal cell counterparts (e.g. K562, HEL, HL60, and MEL cells), and established or otherwise transformed cells lines derived from all of the foregoing. In addition, the constructs of the present invention may be transferred by various means directly into tissues, where they would stably integrate into the cells comprising the tissues. Further, the constructs containing the DNA sequences of the peptides of the invention can be introduced into primary

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cells at various stages of development, including the embryonic and fetal stages, so as to effect gene therapy at early stages of development.

The described constructs may be administered in the form of a pharmaceutical preparation or composition containing a pharmaceutically acceptable carrier and a physiological excipient, in which preparation the vector may be a viral vector construct, or the like, to target the cells, tissues, or organs of the recipient organism of interest, including human and non-human mammals. The composition may be formed by dispersing the components in a suitable pharmaceutically acceptable liquid or solution such as sterile physiological saline or other injectable aqueous liquids. The amounts of the components to be used in such compositions may be routinely determined by those having skill in the art. The compositions may be administered by parenteral routes of injection, including subcutaneous, intravenous, intramuscular, and intrasternal.

The following non-limiting examples illustrate various aspects and embodiments of the invention and should not be contrived as limiting the scope of the invention.

VI. EXAMPLES

The following materials were used in the examples described below. Soluble IGF-1R was obtained from R&D Systems (Cat. # 391-GR/CF). Insulin receptor was prepared according to Bass *et al.*, 1996. The insulin is either from Sigma (Cat. # I-0259) or Boehringer. The IGF-1 is from PeproTech (Cat. # 100-11). All synthetic peptides were synthesized by Novo Nordisk, AnaSpec, Inc. (San Jose, CA), PeptioGenics (Livermore, CA), or Research Genetics (Huntsville, AL) at >80% purity. The Maxisorb Plates are from Nunc via Fisher (Cat. # 12565347). The HRP/Anti-M13 Conjugate is from Pharmacia (Cat. # 27-9421-01). The ABTS solution is from BioF/X (Cat. # ABTS-0100-04).

Example 1

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A. Construction of Phage Library for Identifying IGF-1R and IR Binding Ligands

The schematic for the peptide library "RAPIDLIB $^{\text{TM}"}$ on filamentous phage is shown in Figure 16. DNA fragments coding for peptides containing 40 random amino acids were generated in the following manner. A 145 base oligonucleotide was synthesized to contain the sequence (NNK)₄₀, where N = A, C, T, or G, and K = G or T. This oligonucleotide was used as the template in a PCR amplification along with two shorter oligonucleotide primers, both of which were biotinylated at their 5' ends. The resulting 190 bp product was purified and concentrated with QIAquick spin columns (QIAGEN, Inc. Valencia, CA), then digested with Sfil and Notl. Streptavidinagarose (GibcoBRL Life Technologies, Inc., Rockville, MD) was added to the digestion mixture to remove the cleaved ends of the PCR product as well as any uncut DNA. The resulting 150 bp fragment was again purified over QlAquick spin columns. The phagemid pCANTAB5E (Amersham Pharmacia Biotech, Inc., Piscataway, NJ) was digested with Sfil and Notl, followed by phosphatase treatment. The digested DNA was purified using a 1% agarose gel followed by QIAEX II (QIAGEN). The vector and insert were ligated overnight at 15°C. The ligation product was purified using QIAquick spin columns (QIAGEN). Electroporations were performed at 1500 v in an electroporation cuvette (0.1 mm gap; 0.5 ml volume) containing 12.5 μg of DNA and 500 μl of TG1 electrocompetent cells (see below). Immediately after the pulse, 12.5 ml of pre-warmed (40°C) 2xYT medium containing 2% glucose (2xYT-G) was added and the transformants were grown at 37°C for 1 h. Cell transformants were pooled, the volume measured, and an aliquot was plated onto 2xYT-G containing 100 g/ml ampicillin (2xYT-AG) plates to determine the total number of transformants.

Sequence analysis of randomly selected clones indicated that 54% of all clones are in-frame (Mandecki *et al.*, 1997). The FLAG sequence (Hopp

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et al., 1988) was incorporated into the library as an immunoaffinity tag as shown in Figure 16.

Another phage library expressing 20mer peptides, was constructed according to a similar procedure. The diversity of the library is 1.1×10^{11} different clones.

B. Preparation of Electrocompetent Cells

To prepare electrocompetent cells, an overnight culture of *E. coli* TG1 cells (F'_traD36 lacl^q Δ (lacZ)M15 proAB) / supE Δ (hsdM-mcrB)₅ r_k m_k McrB') thi Δ (lac-proAB) was diluted to an OD₆₀₀ = 0.05-0.1 in 500 ml 2xYT, then grown at 37°C in 4 liter Ehrlenmyer flasks to an OD₆₀₀ = 0.5-0.6. The culture was poured into pre-chilled centrifuge bottles and incubated on ice for 30 min prior to centrifugation at 2000 x g for 30 min (2°C). The supernatant was poured off and the cell pellet was resuspended in a total of 400 ml of ice cold sterile distilled water. The process of centrifugation and resuspension was repeated 2 times. After the last centrifugation, the pellet was resuspended in a total of 25 ml of ice cold water containing 10% glycerol. The cell suspension was transferred to pre-chilled 35 ml centrifuge bottles, and was then pelleted at 2000 x g for 10 min at 4°C. The cells were then suspended in 0.3 ml of the same 10% glycerol solution, aliquotted into smaller tubes, and snap-frozen on dry ice. The aliquots were stored at -80°C.

To amplify the library, the transformants were inoculated into 4 I of 2xYT-AG medium and allowed to grow until the A_{600} increased approximately 400 times. The cells were pelleted by centrifugation at 3000 x g for 20 min, then resuspended in 40 ml 2xYT-AG to which glycerol was added to a final concentration of 8%. The library was stored at -80°C.

C. Phage Rescue

This process was carried out using the standard phage preparation protocol with the following changes. Five individual recombinant cell libraries, with a total diversity of 1.6×10^{10} , were combined and grown to

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OD₆₀₀ = 0.5 in 2xYT-AG at 30°C with shaking (250 rpm). Helper phage (M13K07) was then added (multiplicity of infection (MOI) = 15), and the cells were incubated for 30 min at 37°C without shaking, followed by 30 min at 37°C with shaking (250 rpm). Following infection, cells were pelleted and the supernatant containing the helper phage was discarded. The cell pellet was resuspended in the initial culture volume of 2xYT-A (no glucose) containing 50 mg/ml kanamycin and grown overnight at 30°C with shaking (250 rpm). The cells from the overnight culture were pelleted at 3000 x g for 30 min at 4°C and the supernatant containing the phage was recovered.

The solution was adjusted to 4% PEG, 500 mM NaCl and chilled on ice for 1 h. The precipitated phage were pelleted by centrifugation at 10,000 x g for 30 min, then resuspended in phosphate-buffered saline (1/100 of the initial culture volume) and passed through a $0.45 \mu m$ filter. The phage were titered by infecting TG1 cells. The phage titer for the 40mer peptide library was 4×10^{13} cfu/ml. The phage titer for the 20mer library was 3×10^{-3} .

To amplify the library, the transformants were inoculated into 4 I of 2xYT-AG medium and allowed to grow until the OD_{600} increased approximately 400 times. The cells were pelleted by centrifugation at 3000 x g for 20 min, then resuspended in 40 ml 2xYT-AG to which glycerol was added to a final concentration of 8%. The library was stored at -80°C.

Example 2:

A. Panning IGF-1R

A standard method was used to coat and block all microtiter plates. The soluble IGF-1R ("sIGF-1R") was diluted to 1 mg/ml in 50 mM sodium carbonate buffer, pH 9.5. One hundred microliters of this solution was added to an appropriate number of wells in a 96-well microtiter plate (MaxiSorp plates, Nunc) and incubated overnight at 4°C. Wells were then blocked with MPBS (PBS buffer pH 7.5 containing 2% Carnation® non-fat dry milk) at room temperature (RT) for 1 h.

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Eight wells were used for each round of panning. The phage were incubated with MPBS for 30 min at RT, then 100 µl was added to each well. For the first round, the input phage titer was 4 x 10¹³ cfu/ml. For rounds 2 and 3, the input phage titer was approximately 10¹¹ cfu/ml. Phage were allowed to bind for 2 to 3 h at RT. The wells were then guickly washed 13 times with 200 µl/well of MPBS. Bound phage were eluted by incubation with 100 µl/well of 20 mM glycine-HCl, pH 2.2 for 30 s. The resulting solution was then neutralized with Tris-HCl, pH 8.0. Log phase TG1 cells were infected with the eluted phage, then plated onto two 24 cm x 24 cm plates containing 2xYT-AG. The plates were incubated at 30°C overnight. The next morning, cells were removed by scraping and stored in 10% glycerol at -80°C. For subsequent rounds of affinity enrichment, cells from these frozen stocks were grown and phage were prepared as described above. A minimum of 72 clones were picked at random from the second, third, and fourth rounds of panning and screened for binding activity. DNA sequencing of the clones revealed the abundance of sequences as summarized in Figure 18. Some of the clones (Figure 19) were frameshifted, that is, the relevant peptide sequence was encoded not in the FLAG frame, but in either frame + 1 or - 1.

20 B. ELISA Analyses of Phage

For phage pools, cells from frozen stocks were grown and phage were prepared as described above. For analysis of individual clones, colonies were picked and phage prepared as described above. Subsequent steps are the same for pooled and clonal phage. Microtiter wells were coated and blocked as described above. Wells were coated with either IGF-1R or a control IgG mAb. Phage resuspended in MPBS were added to duplicate wells (100 µl/well) and incubated at RT for 1 h. The phage solution was then removed, and the wells were washed 3 times with PBS at RT. Anti-M13 antibody conjugated to horseradish peroxidase (Pharmacia) was diluted 1:3000 in MPBS and added to each well (100 µl/well). Incubation was for 1 h at RT, followed by PBS washes as described. Color

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was developed by addition of ABTS solution (100 μ l/well; Boehringer). Color development was stopped by adjusting each well to 0.5% SDS. Plates were analyzed at 405 nm using a SpectraMax 340 plate reader (Molecular Devices Corp., Sunnyvale CA) and SoftMax Pro software. Data points were averaged after subtraction of appropriate blanks. A clone was considered "positive" if the A_{405} of the well was \geq 2-fold over background.

For IC₅₀ determinations in a competitive ELISA, microtiter plates were coated with IGF-1R and blocked as described. Phage were prepared as described. Prior to addition of phage to plates, the peptide or recombinant variable antibody or fragment ("rVab"), or an appropriate control, was diluted in PBS and added to duplicate wells (100 µl/well). After incubation for 1 h at RT, the prepared phage were added to each well (100 µl/well) without removing the peptide or rVab solution. After incubation for 1 h at RT, the wells were washed and the color developed as described above.

The clones were next analyzed for binding to the receptor's active site (Figures 20A and 20B). Competitions of phage binding were done with the cognate ligand (i.e., IGF-1). All four phage clones tested, B6, F6, C6 and E5, bound to same site as IGF-1 since the binding of the clones to the immobilized IGF-1R could be inhibited with IGF-1.

To determine the rank order for phage peptides, the human IGF-1R (25 g/ml) was immobilized onto a CM-5 (BIAcore) sensor chip using amino coupling chemistry and the manufacturer's recommended protocol. The final surface density was 1000 RU. A monoclonal antibody was immobilized onto another flow cell as a control surface. Phage were directly injected (30-100 μ l) with a buffer flow rate of 1 μ l/min. Background binding to the control surface was subtracted prior to further analysis.

C. Phage Sequence Analysis

Sequence analysis of several clones shows that there are two distinct populations, designated as Class 1 (Formula motif 2) and Class II (Formula motif 1; Figure 21). Several of these have been chemically synthesized for subsequent testing. Class I peptides contain the consensus sequence D-x-

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F-Y-x-x-L-s-x-L, and are shown to be antagonistic in cell-based assays (Figure 22). Class II peptides contain the consensus N-F-Y-D-W-F-V, and are shown to be agonistic in cell-based assays (Figure 23). Neither of these consensus sequences have any significant linear sequence similarities greater than 2 or 3 amino acids with mature IGF-1.

Example 3: Assays with Synthetic Peptides

Four synthetic peptides, 5.1, 5.2, 5.3 and 5.4 (Figure 21) were made to study the properties of the artificial peptide ligands from phage display.

Synthetic peptides were obtained from a commercial supplier (Anaspec). The peptides were supplied greater than 90% pure by HPLC. The molecular weights of the peptides as determined by mass spectroscopy agreed with the expected values.

IGF-1R (100 μ g/ml) was immobilized onto one flow-cell of a CM-5 sensor chip (Biosensor) using amine coupling chemistry and the manufacturer's recommended protocol. An unrelated IgG was immobilized in the same manner to another flow cell of the same chip as a control surface. Increasing concentrations of synthetic peptide were injected over both surfaces, and the binding responses were allowed to come to equilibrium. After subtraction of background binding from the control surface, the results were used to derive an equilibrium dissociation constant using Scatchard analysis (Figure 24A).

In another experiment, IGF-1R (100 μ g/ml) was immobilized onto a CM-5 sensor chip as described above, and an unrelated IgG was immobilized in the same manner to another flow cell of the same chip. IGF-1 alone, peptide 5.1 alone (corresponding to the B6 phage clone), or different mixes of the two, were injected over the derivatized chip surfaces. The results shown in Figure 24B indicate that the 5.1 peptide inhibits the binding of IGF-1, and the inhibition is increased by increasing amounts of the peptide. The results support the idea of an overlap of the peptide 5.1 binding site and the IGF-1 binding site on IGF-1R.

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Example 4: Construction of Secondary Phage Libraries

Two phage libraries were designed on the basis of the sequences of the Class II binders known to possess agonistic properties in cell-based assays. The goal was to bring the affinity into a range that would allow the peptide to be used in a receptor binding assay and tested in a cell based assay for activity. Among several available mutagenesis methods, we chose one based on gene synthesis and phage display. In this method a library of doped oligonucleotides carrying several mutations in any single DNA molecule is used to obtain a pool of mutant genes, the expression products of which are phage displayed.

A. Phage Library A6L

The approach used was the doped synthesis of the oligonucleotide encoding the sequence of the peptide. The sequence encoding the peptide and the sequence of the synthetic oligonucleotide made are shown in Figures 25A-25B. The amino acid residues belonging to the consensus sequence were kept constant and were not mutated. The ratio of nucleosides in each condensation was chosen to provide an average of 6 nucleotide sequence changes at the DNA level and 4-5 mutations at the amino acid level over the length of the peptides. The regions corresponding to the FLAG, *Sfil* and *Notl* sites were not mutated.

The DNA sequence encoding the A6 peptide was optimized for *E. coli* codon usage by replacing a total of 24 nucleotides as shown in Figure 25A. The TAG stop codons (suppressed in the TG1 *E. coli* strain used) were replaced with CAG (glutamine). Then, the oligonucleotide sequence was designed to include doped nucleosides at positions corresponding to the coding region for the A6 peptide, except for the consensus NFYDWFV (Figure 25A). This synthetic oligonucleotide (Figure 25B) was then used as a template in a PCR reaction. The product of this PCR reaction was then purified, cut with *Sfil* and *Notl* restriction enzymes and cloned into the

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I. Use of the Peptides Provided by this Invention

The IR and IGF-1R agonist and antagonist peptides provided by this invention are useful as potential therapeutics in pharmaceutical compositions, lead compounds for identifying other more potent or selective therapeutics, assay reagents for identifying other useful ligands by, for example, competition screening assays, and as research tools for further analysis of IR and IGF-1R. In particular, the peptide sequences provided by this invention can be used to design secondary peptide libraries, which include members that bind to Site 1 and/or Site 2 of IR or IGF-1R. Such libraries can be used to identify sequence variants that increase or modulate the binding and/or activity of the original peptide at IR or IGF-1R.

IR agonist amino acid sequences provided by this invention are useful as insulin analogs and may therefore be developed as treatments for diabetes or other diseases associated with a decreased response or production of insulin. For use as an insulin supplement or replacement, preferred amino acid sequence are: FHENFYDWFVRQVSK (D117, H2C),

DYKDFYDAIQLVRSARAGGTRDKK (D118, 20E2), KDRAFYNGLRDLVGAVYGAWDKK (D119, 20C11), DYKDLCQSWGVRIGWLAGLCPKK (D116, JBA5),

DYKDVTFTSAVFHENFYDWFVRQVSKK (D113, H2), and GRVDWLQRNANFYDWFVAELG (S175). More preferred IR agonists are: FHENFYDWFVRQVSK (D117, H2C) and GRVDWLQRNANFYDWFVAELG (S175). Most preferred is GRVDWLQRNANFYDWFVAELG (S175). Preferred dimer sequences are represented by S170, S171, S172, S232, S300 sequences (see Table 15).

IGF-1R antagonist amino acid sequences provided by this invention are useful as treatments for cancers, including, but not limited to, breast and prostate cancers. Human and breast cancers are responsible for over 40,000 deaths per year, as present treatments such as surgery,

chemotherapy, radiation therapy, and immunotherapy show limited success.

The IGF-1R antagonist amino acid sequences disclosed herein are also

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useful for the treatment or prevention of diabetic retinopathy. Recent reports have shown that a previously identified IGF-1R antagonist can suppress retinal neovascularization, which causes diabetic retinopathy (Smith *et al.*, 1999).

IGF-1R agonist amino acid sequences provided by this invention are useful for development as treatments for neurological disorders, including stroke and diabetic neuropathy. Reports of several different groups implicate IGF-1R in the reduction of global brain ischemia, and support the use of IGF-1 for the treatment of diabetic neuropathy (reviewed in Auer *et al.*, 1998; Apfel, 1999).

J. Methods of Administration

The amino acid sequences of this invention may be administered as pharmaceutical compositions comprising standard carriers known in the art for delivering proteins and peptides and by gene therapy. Due to the labile nature of the amino acid sequences parenteral administration is preferred. Preferred modes of administration include aerosols for nasal or bronchial absorption; suspensions for intravenous, intramuscular, intrasternal or subcutaneous, injection; and compounds for oral administration. Other modes of administration and examples of suitable formulative components for use with this embodiment are discussed below. Other modes of administration include intranasal, intrathecal, intracutaneous, percutaneous, enteral, and sublingual. For injectable administration, the composition is in sterile solution or suspension or may be emulsified in pharmaceutically- and physiologically-acceptable aqueous or oleaginous vehicles, which may contain preservatives, stabilizers, and material for rendering the solution or suspension isotonic with body fluids (i.e. blood) of the recipient. Excipients suitable for use are water, phosphate buffered saline, pH 7.4, 0.15 M aqueous sodium chloride solution, dextrose, glycerol, dilute ethanol, and the like, and mixtures thereof. Illustrative stabilizers are polyethylene glycol. proteins, saccharides, amino acids, inorganic acids, and organic acids. which may be used either on their own or as admixtures. The amounts or

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quantities, as well as routes of administration, used are determined on an individual basis, and correspond to the amounts used in similar types of applications or indications known to those of skill in the art.

The constructs as described herein may also be used in gene transfer and gene therapy methods to allow the expression of one or more amino acid sequences of the present invention. Using the amino acid sequences of the present invention for gene therapy may provide an alternative method of treating diabetes which does not rely on the administration or expression of insulin. Expressing insulin for use in gene therapy requires the expression of a precursor product, which must then undergo processing including cleavage and disulfide bond formation to form the active product. The amino acid sequences of this invention, which possess activity, are relatively small, and thus do not require the complex processing steps to become active. Accordingly, these sequences provide a more suitable product for gene therapy.

Gene transfer systems known in the art may be useful in the practice of the invention. Both viral and non-viral methods are suitable. Examples of such transfer systems include, but are not limited to, delivery via liposomes or via viruses, such as adeno-associated or vaccinia virus. Numerous viruses have been used as gene transfer vectors, including papovaviruses (e.g., SV40, adenovirus, vaccinia virus, adeno-associated virus, herpes viruses, including HSV and EBV, and retroviruses of avian, murine, and human origin). As is appreciated by those in the art, most human gene therapy protocols have been based on disabled murine retroviruses. Recombinant retroviral DNA can also be employed with amphotrophic packaging cell lines capable of producing high titer stocks of helper-free

A recombinant retroviral vector may contain the following parts: an intact 5' LTR from an appropriate retrovirus, such as MMTV, followed by DNA containing the retroviral packaging signal sequence; the insulator element placed between an enhancer and the promoter of a transcription

recombinant retroviruses (e.g., Cone and Mulligan, 1984).

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unit containing the gene to be introduced into a specific cell for replacement gene therapy; a selectable gene as described below; and a 3' LTR which contains a deletion in the viral enhancer region, or deletions in both the viral enhancer and promoter regions. The selectable gene may or may not have a 5' promoter that is active in the packaging cell line, as well as in the transfected cell.

The recombinant retroviral vector DNA can be transfected into the amphotrophic packaging cell line Ψ-AM (see Cone and Mulligan, 1984) or other packaging cell lines which are capable of producing high titer stocks of helper-free recombinant retroviruses. After transfection, the packaging cell line is selected for resistance to G418, present at appropriate concentration in the growth medium. Adenoviral vectors (e.g. DNA virus vectors), particularly replication-defective adenovirus vectors, or adeno-associated vectors, have been described in the art (Kochanek *et al.*, 1996; Ascadi *et al.*, 1994; Ali *et al.*, 1994).

Nonviral gene transfer methods known in the art include chemical techniques, such as calcium phosphate co-precipitation, direct DNA uptake and receptor-mediated DNA transfer, and mechanical means, such as microinjection and membrane fusion-mediated liposomal transfer. In addition, viral-mediated gene transfer can be combined with direct *in vivo* gene transfer using liposomes, thereby allowing the delivery or the viral vectors to tumor cells, for example, and not to surrounding non-proliferating cells. A description of various liposomes which are stated as being useful for transferring DNA or RNA into cells is present in United States Patents 5,283,185 and 5,795,587. The retroviral vector producer cell line can also be injected directly into specific cell types, e.g., tumors, to provide a continuous source of viral particles, such as has been approved for use in patients afflicted with inoperable brain tumors.

Receptor-mediated gene transfer methods allow targeting of the DNA in the construct directly to particular tissues. This is accomplished by the conjugation of DNA (frequently in the form of a covalently closed supercoiled

pCANTAB5E vector as described for the original peptide library. Over 10¹⁰ different clones were obtained in the final library.

B. Phage Library A6S

While the consensus sequence NFYDWFV was kept constant in the A6S library, the flanking regions were randomized in the A6S library as shown in Figure 26A. The codons in the random region were of the NNK type to reduce the frequency of stop codons (N = A, C, G, or T; K = G or T). The sequence of the synthetic oligonucleotide made is given in Figure 26B. This synthetic oligonucleotide was then used as a template in a PCR reaction. The product of this PCR reaction was then purified, cut with *Sfi* I and *Not* I restriction enzymes and cloned into the pCANTAB5E vector as described for the original peptide library. Over 10⁹ different clones were obtained in the final library.

C. Secondary Phage Library Based on Clone H5

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Peptide H5 (LCQRLGVGWPGWLSGWCA) was identified in an independent experiment as a binder to the rat growth hormone binding protein. This peptide and four other H5-like peptides, including 2C3-60 (Figure 27), were found in cell culture experiments to possess agonistic activity toward IGF-1R $^+$ cells, but not against IGF-1R $^-$ cells. Further, subsequent *in vitro* experiments showed that the H5-like peptides are not competed by IGF. This suggests that these peptides recognize a second allosteric site on IGF-1R. BIAcore analysis showed that binding of the 2C3-60 peptide to IGF-1R is ~20 μ M. Subsequently, a phage library of mutants of the H5 sequence was constructed and used for panning against IGF-1R.

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Gene synthesis to introduce mutations and phage display were used to construct an H5 secondary library. In this method a library of doped oligonucleotides carrying several mutations in any single DNA molecule is used to obtain a pool of mutant genes which are phage displayed. This method allowed the encoding of both the original H5 peptide as control as

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well as versions containing high numbers of mutations per peptide in a very large library (>10¹⁰).

Therefore, the H5 secondary mutant library was designed to contain an average of four amino acid changes (mutations) per peptide. The number of possible mutant H5 peptide sequences having four mutations is 1.0×10^{10} and is equivalent to the actual size of the secondary phage library. Sequence analysis indicates that of these peptides 30% have 3-4, 33% have 1-2 and 32% have 5-6 mutations. There also was a small percent with 7-8 mutations and 5% clones without any mutation.

An oligonucleotide based on the DNA sequence encoding the H5 peptide was synthesized. The sequence of the oligonucleotide is: 5'-CTACAAAGACCTGTGTTAGAGTTTGGGGGTTACGTATCCGGGTTGGT TGGCGGGGTGGTGTGCGGCGGCCGCAGTGTGA-3'

The underlined base positions were synthesized as mixtures of four nucleosides as follows:

 \underline{A} = 90% A; 3.3% C; 3.3% G; and 3.3% T \underline{C} = 3.3% C; 90% C; 3.3% G; and 3.3% T \underline{G} = 3.3% C; 3.3% C; 90% G; and 3.3% T \underline{T} = 3.3% C; 3.3% C; 3.3% G; and 90% T

20 Using this oligonucleotide as a template, the H5 secondary library was constructed, electroporated, amplified, and rescued essentially as described for the original peptide library. The final diversity of this secondary library was ~10¹⁰.

D. Characterization of Libraries

25 Forty-eight randomly picked clones from each of the secondary libraries (Round 0, before panning) were rescued and the phage was assayed in an ELISA for binding to the anti-E-tag mAb, as well as for binding to IGF-1R (E-tag is used as an indicator of expression of displayed peptides on phage surfaces). The results showed that although most of the clones in

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the two libraries (70%) display a peptide (i.e., are positive for E-tag), only about 6% of the clones from the A6 long (A6L) library bind to IGF-1R by phage ELISA, and none of the 24 clones tested from the A6 short (A6S) library bind to IGF-1R. This indicates that the most common outcome of random mutagenesis is the loss of IGF-1R affinity. Nevertheless, some mutants do retain their binding properties and some have improved affinities (see below).

E. Panning with the Secondary Libraries

The two secondary libraries of Example 4 were used in a panning experiment against IGF-1R. Approximately 50 clones from each four rounds of panning were analyzed in a phage ELISA to identify the clones that bind to the receptor. The positive clones were subjected to DNA sequencing and protein sequence comparison. Figure 28 provides a listing of different sequences obtained from panning with the A6S library. The results show that a variety of phage peptide sequences can bind to IGF-1R, while the consensus sequence NFYDWFV is preserved in the majority of instances.

The H5 secondary phage library was panned against IGF-1R to find H5-like peptides with higher affinities for IGF-1R

The H5 Library has a diversity of \sim 2.6 x 10^{10} clones with a phage titer of 1.0×10^{13} phage ml⁻¹. A total of three rounds of panning were performed. Table 2 summarizes the results from the three rounds of panning and shows the ELISA results for the individual clones selected from each round, the number of clones examined in each round of panning, as well as the number and percentage of E-Tag⁺ clones and IGF-1R⁺ clones.

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		E-Ta	ıg ^{+, a}	IGF-1R ^{+, b}		
Round	Total	Number	%	Number	% Total	
0	32	22	69 %	0	0 %	
1	128	116	91 %	1	1 %	
2	128	108	84 %	2	2 %	
3	160	116	91 %	65	51 %	

F. TABLE 2: Results of panning with the H5 secondary phage library.

^aE-Tag⁺ means ELISA absorbance values >2X background. ^bIGF-1R⁺ means ELISA Absorbance >2X background. Background absorbance values are 0.05 to 0.075.

Each of the IGF-1R⁺ clones were sequenced, as were 15 IGF-1R⁻ clones with high E-Tag values (Absorbance >1.0). These sequences are shown in Figure 29. There is no discernible difference between binding sequences and the non-binding sequences with the exception that all of the binding sequences hold the Gly at position 6 constant. All sequences, binding and non-binding, hold the TAG stop codon constant at position 3 (the *E. coli* strain used in phage production contains the supE44 mutation, therefore Gln replaces the TAG and it denoted in Figure 29 by $\underline{\mathbf{Q}}$). This suggests TAG stop codon is required for phage production and not binding.

15 Example 5: Construction of the rVab Recombinant Antibody Variable Region library

The design, expression and purification of single-chain antibodies has been reviewed (Rader and Barbas, 1997; Hoogenboom, 1997). Briefly, the variable portion of the heavy chain (V_H) is linked to the variable portion of the light chain (V_L) by a flexible peptide linker. Random combinations of V_H and V_L genes can be genetically combined to provide some of the diversity required for a library of recombinant variable region antibodies (rVabs) (Figure 30). In our library, further diversity is provided by full randomization

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of the 6-12 amino acids comprising the V_{H} CDR3 (indicated as "D" in Figure 30).

A total of 49 human genomic v_h genes and ten human genomic v_l genes (Figure 31) were isolated from total human genomic DNA by PCR. The other genetic components of the library (V_h , CDR3, j_h , linker, and j_l) were derived from synthetic oligonucleotides. Assembly of these components was done using directional cloning as outlined in Figure 32 and Figure 33.

A. Ligations

The general schematic for the assembly of the rVab library ("GRABLIB^{TM")} is provided in Figure 30. Four gene fragments (VH, VHCDR3/JH/LINKER, VL and JL) were ligated together in the proper orientation and cloned into pCANTAB 5E (Pharmacia). Directional cloning was achieved using the BsrDl restriction enzyme (Figure 32). Forty-nine germline VH segments and ten VL segments encoding many of the genes from the human VH and VL repertoire were isolated (Figure 31) using the polymerase chain reaction. VH CDR3 (ranging from 6 to 12 amino acids) /JH/Linker fragments were generated by ligation of four oligonucleotides (WM 2.1, 2.2, 2.3 and 2.4) and cloning the resulting fragment into the plasmid pUC18 previously cut with Kpnl and Hindlll. The insert was then amplified using PCR and oligonucleotide primers to introduce a synthetic Dsegment of 6 to 12 amino acids having a random sequence and the BsrDl restriction site. The JL gene fragments were assembled as a result of annealing of two synthetic oligonucleotides. The assembled fragments (200 ng) were used as template in a PCR amplification along with two shorter oligonucleotide primers, both of which were biotinylated at their 5' ends. The resulting 800 bp product was purified and concentrated with QIAquick spin columns (QIAGEN), then digested with the Sfil and Notl restriction enzymes. Streptavidin-agarose (GibcoBRL) was added to the digestion mixture to remove the cleaved ends of the PCR product as well as any

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uncut DNA. The resulting 800 bp fragment was purified by passing DNA over QIAquick spin columns.

Phagemid pCANTAB 5E (Pharmacia) was digested with the Sfil and Not! restriction enzymes, which was followed by the alkaline phosphatase treatment to dephosphorylate the ends of the restriction fragments generated. The digested DNA was purified by running the digested plasmid DNA on a 1% agarose gel, followed by the DNA purification using the QIAEX II (QIAGEN) column. The vector and insert DNA were ligated overnight at 16°C. The ligation product was purified using QlAquick spin columns (QIAGEN) and electroporations were performed at 1500 v in a electroporation cuvette (0.1 mm gap; 0.5 ml volume, BTX, Inc.). The amount of DNA in one electroporation was 12.5 µg per 500 µl of TG1 electrocompetent cells. Immediately after the pulse, 12.5 ml of a prewarmed (40°C) 2xYT medium containing 2% glucose (2xYT-G) was added, and the transformants were grown at 37°C for 1 h. The transformants were pooled, the volume measured, and an aliquot was plated onto the 2xYT-G medium containing 100 µg/ml ampicillin (2xYT-AG) plates to determine the total number of transformants. The number of different transformants and the diversity of the library was 3×10^{10} .

The electrocompetent cell preparation, phage library amplification, library phage rescue, phage preparations and coating of microtiter plates were done as described above for the peptide library.

B. Panning for IGF-1R Binders with rVab Antibody Library

1. Panning Procedure

Panning of the antibody library was done essentially as described for the peptide library, for a total of four rounds. Of the 200 clones tested, approximately 10% bound specifically to sIGF-1R. Among these specific binders, 40% can be competed by IGF-1 for receptor binding. The clonal analysis and DNA sequencing (Figures 31-39) followed by ELISA and cell-based assays (Figures 40-46) have shown that two clones, 43G7 and M100,

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are agonistic with ED $_{50}$ values of approximately 20 nM (a plot for the 43G7 antibody is shown in Figure 41). Two other rVabs, 1G2P and 39F7, have been shown to be antagonistic, with IC $_{50}$ values of approximately 20 nM (Figure 42).

Microtiter wells were coated with IGF-1R as described above, with eight wells being used for each round of panning. The phage were incubated with MPBS for 30 min at RT, then 100 μ l of the phage suspension was added to each well. For the first round, the input phage titer was 8 x 10^{13} cfu/ml. For rounds 2 and 3, the input phage titer was approximately 10^{11} cfu/ml. Phage were allowed to bind for 2 to 3 h at RT. The wells were then quickly washed 13 times with 200 μ l /well of MPBS. Bound phage were eluted by incubation with 100 μ l/well of 20 mM glycine-HCl, pH 2.2 for 30 s. The resulting solution was then neutralized with Tris-HCl, pH 8.0. Log phase TG1 cells were infected with the eluted phage, then plated onto two 4 cm x 4 cm plates containing 2XYT-AG. The plates were incubated at 30°C overnight. The next morning, cells were removed by scraping and stored in 10% glycerol at -80°C. For subsequent rounds of affinity enrichment, cells from these frozen stocks were grown and phage were prepared as described above.

20 2. Elisa Analyses Of Phage Pools

To prepare the phage pools, cells from frozen stocks were grown and phage were prepared as described above. Microtiter wells were coated and blocked as described above. The wells were coated with either IGF-1R (R&D Systems, Inc.) or with control BSA. Phage resuspended in MPBS were added to duplicate wells (100 μ l/well) and incubated at RT for 1 h. The phage solution was then removed, and the wells were washed 3 times with PBS at RT. Anti-M13 antibody conjugated to horseradish peroxidase (Pharmacia) was diluted 1:3000 in MPBS and added to each well (100 μ l/well). Incubation was for 1 h at RT, followed by PBS washes as described. Color was developed by addition of ABTS solution (100 μ l/well;

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Boehringer). Color development was stopped by adjusting each well to 0.5% SDS. Plates were analyzed at 405 nm using a SpectraMax 340 plate reader (Molecular Devices) and SoftMax Pro software. Data points were averaged after a subtraction of appropriate blanks. Phage pools was considered "positive" if the A_{405} of the well was > 2-fold over background.

3. Competition ELISAs

For IC₅₀ determinations, microtiter plates were coated with IGF-1R and blocked as described. Phage and soluble rVabs were prepared as described above. Prior to addition of phage or soluble rVabs to the plates, IGF-1 solution in PBS (1 μ g/ml) was added to duplicate wells (100 μ l/well). After incubation for 1 h at RT, the prepared phage were added to each well (100 μ l/well) without removing the IGF-1 solution. After incubation for 1 h at RT, the wells were washed and the color was developed as described above.

Six rVab clones bound specifically to IGF-1R The sequences of the clones are shown in Figure 34-39.

4. Expression And Purification Of Soluble rVabs

 $E.\ coli$ HB2151 carrying the rVab genes on the pCANTAB5E plasmid (Pharmacia) were grown in 2xYT supplemented with 100 μg/ml ampicillin and 1% glucose at 37° C overnight and then subcultured in the absence of glucose at an OD600 of 0.1, and grown at 21° C until OD600 was 1.0. Expression was induced by the addition of IPTG to 1 mM and the cells were grown for 16 h at 30° C. The cells and culture supernatant were separated by centrifugation and samples of the cell pellet and supernatant were analyzed on a 15% SDS-PAGE gel followed by the Western blot analysis using the mouse monoclonal antibody anti-E-Tag-HRP conjugate (Pharmacia) to visualize the expressed product. The expressed rVabs were purified from the supernatant by precipitation with ammonium sulphate (which was added to 70% saturation) at 21° C, followed by centrifugation at

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10,000 g for 15 min. The aqueous phase was discarded, and the pellet resuspended and dialyzed in PBS (phosphate buffered saline, pH 7.4) at 4° C overnight. Insoluble material was removed by centrifugation at 10,000 g, and the supernatant was filtered through a 0.22 μ m membrane and purified on an anti-E-Tag antibody affinity column (Pharmacia). The affinity resin was equilibrated in TBS (0.025 M Tris-buffered saline, pH 7.4) and the bound protein was eluted with the Elution buffer (100 mM glycine, pH 3.0). The rVab was concentrated to 1 mg/ml, dialyzed against TBS and stored at 4° C. The SDS-PAGE, Western blot analysis and N-terminal sequence analysis of the affinity purified material were performed according to standard protocols.

5. Size Exclusion FPLC Chromatography

The affinity purified rVabs were fractionated by size exclusion FPLC on a Superdex 75 HR10/30 column (Pharmacia) to determine the molecular size and aggregation state of the rVabs. For calibration of the column, High and Low Molecular Weight Gel Filtration Calibration Kits (Pharmacia) were used. Fractions from several chromatographic separations corresponding to a molecular weight of 30 kDa were pooled and concentrated to 0.7-1.0 mg/ml using Amicon XM10 membranes. Protein concentrations were determined using the BCA protein assay kit (Pierce Chemical Co., Rockford, IL).

6. BIAcore Analyses

IGF-1R was immobilized onto one flow cell of a CM-5 sensor chip (Biosensor) using amine coupling chemistry and the manufacturer's recommended protocols. BSA was immobilized in the same manner to another flow cell of the same chip as a control surface. Increasing concentrations of the affinity-purified rVabs were injected over both surfaces, and the binding responses were allowed to come to equilibrium. After a subtraction of the background binding (from the control surface), the equilibrium dissociation constant was derived using Scatchard analysis.

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7. Time-Resolved Fluorescence Assay

We have selected the basic format of an *in vitro* competitive receptor binding assay as the basis of a heterogeneous screen for small organic molecular replacements for IGF-1. In the present assay, occupation of the active site of IGF-1 receptor is quantified by time-resolved fluorometric detection (TRFD) with streptavidin-labeled europium (saEu) complexed to biotinylated peptides (bP). In this assay, saEu forms a ternary complex with bP and IGF-1 receptor (i.e., IGF-1R:bP:saEu complex). The TRFD assay format is well established, sensitive, and quantitative (Tompkins *et al.*, 1993). We demonstrate the assay using 43G7 rVab or a biotinylated peptide. Furthermore, we show that both assay formats faithfully report the competition of the biotinylated ligands binding to the active site of IGF-1R by IGF-1.

In these assays, soluble IGF-1 receptor is coated on the surface of microtiter wells, blocked by PBS containing milk and BSA, and then incubated with biotinylated peptide or rVab. Unbound bP is then washed away and saEu is added to complex with receptor-bound bP. Upon addition of the acidic enhancement solution, the bound europium is released as free Eu⁺³ which rapidly forms a highly fluorescent and stable complex with components of the enhancement solution. The IGF-1R:bP bound saEu is then converted into its highly fluorescent state and detected by TRFD.

a. Preparation of [Eu3+]-Labeled rVab 43G7

One milligram of rVab 43G7 (the sequence is provided in Figure 34) was added to 300 nmol Eu³+-chelated N¹(P-isothiocyanatobenzyl)-diethylenetriamine-N¹,N²,N³-tetracetic acid (Wallac). The reaction was conducted at pH 8.5. The tube was mixed gently and placed at ambient temperature. When the reaction was complete (16 h), the sample was diluted 10-fold into the Tris-buffered saline (TBS), pH 7.5, and the separation of the labeled rVab from the unlabeled rVab and free-Eu³+ was achieved by using the PD-10 column. The protein concentration and

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labeling efficiency were determined using a Europium standard solution (Wallac).

b. Assay Method

IGF-1R (5 μg/ml in 50 mM NaHCO₃) was coated onto low-fluorescence MaxiSorp (Nunc) plates (100 μl/well) overnight at 4°C. The plates were blocked with PBS containing 2% non-fat milk and 0.05% BSA for 2 h at RT, followed by three PBS washes. For competitive ELISA, serial dilutions of unlabelled IGF-1 (0.1 nM-100 μM) were added to the plates (100 μl/well) and incubated at RT for 1-2 h. 100 μl [Eu3⁺] rVab 43G7 in Wallac's DELFIA assay buffer (100 mM Tris-HCl, pH 7.8; 150 mM NaCl; 0.5% BSA, 0.05% bovine lg; 0.05% NaN₃; 0.01% Tween-20) was added and incubated for 1.5 h at RT. The plates were then washed 5 times with TTBS (TBS buffer containing Tween-20; Wallac) and tapped dry. Subsequently, 100 μl of DELFIA enhancement solution (100 mM acetone-potassium hydrogen phtalate, pH 3.2; 15 mM 2-naphtyltrifluoroacetate; 50 mM tri(n-octyl)-phosphine oxide; 0.1% Triton X-100) was added to each well, and the plates were shaken for 10 min at RT. Fluorescence of each sample well was measured at 615 nm using a DELFIA 1234 fluorometer (EG&G Wallac).

The dose response of TRFD of Eu was studied in microtiter wells. Detection is linear over the range 0.2 to 200 fmol with a limit of detection (twice background) of 0.05 fmol. There are 6010 fluorescent units (FU) per fmol of Eu. Binding and detection of Eu-SA, (4.7 mol Eu/mol streptavidin) to wells coated with biotinylated BSA (bBSA) (6 mol biotin/mol BSA) is linear over the entire range tested. The specific fluorescent activity of streptavidin Eu-SA (with 4.7 mol Eu/mol SA) is 28 kfu/fmol and the limits of detection (i.e., twice background) are 0.030 fmol. Coating with IGF-1R was linear up to inputs of 200 ng/well and thereafter appeared to saturate at about 660 ng bIGF-1 (biotinylated IGF-1) per well. This is the expected amount based on the manufacturer's information about protein saturation densities of these wells (Nunc manual). These studies show a limit of detection of bIGF-1 (i.e.,

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twice background) of 0.05 fmol bIGF-1. The ability of this assay format to detect specifically bound bIGF-1 (or bPeptides) to IGF-1R coated wells was determined.

8. Elisa Analyses

ELISA was performed on selected rVabs. We found that the native IGF-1 ligand inhibits the binding of peptide 5.1 (the sequence of which originates from the phage clone B6) as shown in Figure 43. The detection of the peptide involved a sandwich configuration with the Eu-labeled streptavidin. It was determined that the binding of Eu-labeled rVab 43G7 to IGF-1R is inhibited by IGF-1 with an IC₅₀ of approximately 2 nM, as shown in Figure 44. The binding of the biotinylated peptide 5.1 is inhibited by rVab 43G7 with an IC₅₀ of about 10 nM (Figure 45), indicating that both the peptide and rVab bind to the same site on the IGF-1R molecule.

Figures 46A-46D demonstrates the binding properties of the 43G7 antibody. The binding of the Eu-labeled 43G7 antibody is competed by peptide 5.1 (clone B6) (Figure 46A) and by the non-labeled 43G7 (Figure 46B), as well as by rVab 39F7 (Figure 46C) and rVab 1G2P (Figure 46D). The sequences of rVabs 1G2P and 39F7 are provided in Figure 35 and Figure 36, respectively.

20 C. Conclusions

The above results support the use of this assay procedure as a high throughput screen for agents, with affinities for sites on the human IGF-1R which bind IGF-1. The studies show the IGF-1-specific peptides bind in a dose-dependent, saturable manner and are blocked from binding by agents known to bind to the active site of the receptor. This competition is reproducible and easily quantified. Furthermore, the TRFD assay, which is automatable, is much more sensitive than is an ELISA.

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Example 6: Agonistic and Antagonistic Activity of IGF-1R-Binding Peptides

Agonistic and antagonistic activities of the IGF-1-specific peptides were tested in FDCP2 cells (NIH) which express IGF-1R. The cell line requires either IL-3 or IGF-1 for growth, and the cells were maintained in RPMI 1640 medium containing 15% FCS (fetal calf serum). Agonism activity assays were performed in a total volume of 100 μl in 96 well plates (flat bottom). Cells were seeded at 30,000 cells/well in 50 μl RPMI 1640 (without IL-3) medium containing 15% FCS in triplicate wells. To each well, 50 μl of a solution containing either IGF-1, rVabs or peptides at different concentrations was added, followed by incubation for 42 h in a CO₂ incubator at 37°C.

Assays to measure the antagonistic activity were performed in a total volume of 100 µl in 96 well plates. An IGF-1-specific peptide, rVab or an appropriate control was added to wells containing 0.003 µM of human IGF-1 and incubated at 37°C for 18 h in CO₂ incubator. Proliferation assays were performed using WST-1 reagent. The WST-1 tetrazolium salt (slightly red) is cleaved to formazan (dark red) by the succinate-tetrazolium reductase system, which is active only in viable cells. An increase in the number of cells results in an increase in the overall activity of the dehydrogenase which results in a higher absorbance at 450 nm. Ten microliters of WST-1 reagent was added to each well and the plates incubated for 1-4 h at 37°C. Proliferation was measured by absorbance at 450 nm. Both 5.3 and 5.4 peptides showed an agonistic activity at the 10 µM concentration (Figure 23). Peptides 5.1 and 5.2 showed a significant antagonistic activity in the 3-30 µM concentration range (Figure 22). Control peptide showed no antagonistic activity at the concentrations tested.

The results described demonstrate the feasibility of both the chemical synthesis of and construction of a recombinant expression vector to make sufficient soluble peptide (free or as fusion with some carrier protein) or rVab for testing agonist and antagonist activities. The results provide peptide-

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receptor pairs to be used in a site directed competition binding assay wherein IGF-1R can be used as one member of the pair, with the peptide or a rVab as the other member. Labeling of each member, and detection of pair formation, using either member in radioactive or nonradioactive labeled forms, is possible by a variety of methods known to those skilled in the art of building competition binding assays. This assay provides a high throughput screening assay to identify small organic molecules which bind to the active site of IGF-1R.

Example 7: Phage Library B6-2

This library was designed based on the "core" sequence of the Class I binders Site 1(B6) which posses antagonistic activities in a cell proliferation assay. The core sequence was determined as DPFYHKLSEL, where the residues F (position 3, X_6 of Formula 2), Y (position 4, corresponding to X_7 of Formula 2), L (position 7, corresponding to X_{10} of Formula 2) and L (position 10, corresponding to X_{13} of Formula 2) were the only residues observed at those positions. The purpose of this library was to test the possibility that some binders will show deviations from the core sequence, especially at the positions where substitutions had not previously been observed. The library was therefore made from doped oligonucleotides so that, on average, half of the amino acid residues were altered per peptide. The library was made as described in the original B6 library, i.e., synthetic oligonucleotides were first amplified in a PCR reaction. The resultant products were cloned into pACANTAB5E (Pharmacia) via *Sfi*I and *Not*I restriction sites as previously described. Over 10^{10} different clones were obtained in the final library.

A. Random 20mer Library

1. Panning with the B6-2 and Random 20mer Libraries

The libraries were affinity selected against IGF-1R. 96 clones from round 3 of panning from B6-2 library and 96 clones from round 4 from the

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random 20mer library were analyzed in a phage ELISA to identify binders. The DNA of binders was then determined. The results from both libraries show that positions other than positions 3, 4, 7 and 10 as described above can vary relatively at ease (see tables below), while variability at positions 3, 4, 7 and 10 is much more restricted. The results from the B6-2 library show that the restricted core residues were maintained in all binders except one, which happened only in one instance, L (position 7) can be substituted by another hydrophobic residue, M, at that position. The result from the random 20 library panning revealed that another aliphatic amino acid residue. I can substitute for L at position 7. In addition, the restricted residue at position 10 (L) can also be substituted with amino acid residue M. Thus, 2 of the previously identified restricted residues (L at positions 7 and 10) are not absolute, even though L is preferred at these positions. It should be noted that the failure to observe a substitution at a particular residue position does not necessarily indicate that substitutions cannot be made without losing activity, rather such an absence of substitution is indicative of a preference or an aversion for substitution. The findings are summarized below:

B. Results

Combined results from binding clones isolated from B6-2 (doped core) and random 20 libraries of the Formula 2 motif are shown below in Table 3. Sequences from 25 clones from B6-2 and 29 clones from the random 20mer library were analyzed. Numbers adjacent the amino acid residues represent the frequency with which a specific amino acid was observed at the corresponding position.

TABLE 3

				*	*			*			*	
	B6 CORE	D37	P34	F54	Y54	H12	K15	L46	S16	E27	L53	L30
5		E	A			A4	A10	I7	A5	A 6	M	A6
		G3	D4			D7	G7	M	D3	D3		I
		K4	Ε			E9	I		E4	G2		K
		R2	G10			G	L8		F5	K		S2
		S5	L			K3	МЗ		G4	L		\mathbf{T}
10		Т	Q			L2	N		Η	Q3		V13
		V	S			M	Q		L6	R6		
			${f T}$			N	R5		M	S4		
						Q7	${ m T}$		N	V		
						R4	V		Q2			
15						S	W		R2			
						${ m T}$			T2			
						V			Y2			

Based on the substitutions observed above, the following preferences shown in Table 4 are preferred for substitutions in the amino acid sequence of Formula 2 for binding to IGF-1R.

TABLE 4

	25X98	X99	X6	X7	X8	X9	X10	X11	X12	X13_	X100
ı	1(D)	2(P)	3(F)	4(Y)	5(H)	6(K)	7(L)	8(S)	9(E)	10(L)	11(L)
	no aromatics; no large aliphatics; no c	no aromatics; no c; no + charged			no aromatics; no C; no P; no I	no aromatics, except W; no – charged; no C; no P		no C; no P	no aromatics; no C; no P		no aromatics; no aliphatics; no C; no P

Example 8:

A composite of amino acid residues observed in sequences of random 20mer, 40mer and A6 (Formula 1) clones is illustrated below:

	A6 CORE	NFYD	W F
5		D6	A
		E	E9
		G6	G2
		Н3	Q4
		K	R
10		P	S
		Q8	
		S	
		T	
		V	

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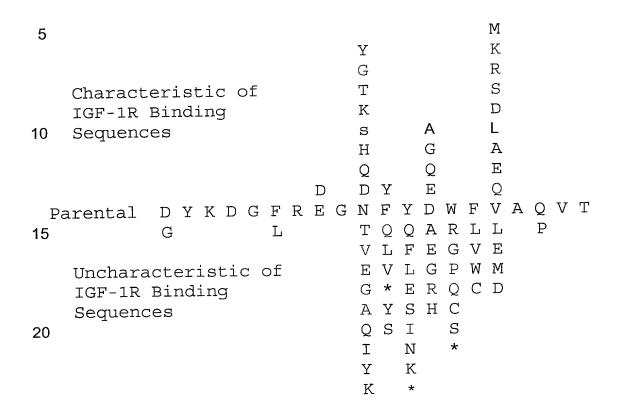
A summary of preferences for A6 residues is shown in Table 5 below. An illustration of residues which are characteristic of IGF-1R binding sequences (above parental sequence) and those which are not typically associated with binding sequences (below parental sequence). Table 6.

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TABLE 5

	X1	X2	X3	X4	X5_
1(N)	2(F)	3(Y)	4(D)	5(W)	6(F)
no aromatics; no large aliphatics; no C; no P			no hydrophobics, except tiny; no C; no P		

TABLE 6



25 Example 9: Panning the Insulin Receptor

A standard method was used to coat and block all microtiter plates. IR (prepared according to Bass *et al.*, 1996) was diluted to 2 μ g/ml in PBS. Fifty microliters of this solution was added to an appropriate number of wells in a 96-well microtiter plate (MaxiSorp plates, Nunc) and incubated overnight at 4°C. Wells were then blocked with a solution of 2% non-fat milk in PBS (MPBS) at RT for at least 1 h.

A. Two-Day Panning Procedure

Eight wells coated with IR were used for each round of panning. One hundred microliters of phage were added to each well. For the first panning round, the input phage titer was 4×10^{13} cfu/ml. For subsequent rounds, the

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input phage titer was approximately 10¹¹ cfu/ml. Phage were allowed to bind for 2-3 h at RT. The wells were then quickly washed 13 times with 300 µl/well of PBS containing 0.5% Tween-20 (PBST). Bound phage were eluted by incubation with 150 µl/well of 50 mM glycine-HCl, pH 2.0 for 15 min. The resulting solution was pooled and then neutralized with Tris-HCl, pH 8.0. An equal volume of log-phase TG1 cells were infected with the eluted phage, then plated onto two 24 cm x 24 cm plates containing 2xYT-AG. The plates were incubated at 30°C overnight. The next morning, cells were removed by scraping and stored in 10% glycerol at -80°C. For subsequent rounds of affinity enrichment, cells from these frozen stocks were grown and phage were prepared as described above. A total of 216 clones from the 20mer library and 120 clones from the 40mer library were picked at random from the third and fourth rounds of panning and screened for IR binding activity. DNA sequencing of the clones revealed the abundance of sequences as summarized in Figures 1A, 1B, 2A, 2C, 10A and 10B.

B. One-Day Panning Procedure

Log phase TG1 cells were infected with the eluted phage, amplified in the 2xYT medium for 1 h at 37°C prior to the addition of helper phage, ampicillin and glucose (2% final concentration). After incubation for 1 h at 37°C, the cells were spun down and resuspended in the 2xYT-AK medium. The cells were then returned to the shaker and incubated overnight at 37°C. The overnight phage was then precipitated and subjected to the next round of panning. A total of 96 clones were picked at random from rounds 3 and 4 and screened for binding activity.

To isolate specific binders, each library was panned against a soluble form of the human IR. This IR is composed of the extracellular domains of both the α and β chains of the natural receptor, as well as the constant domain from immunoglobulin Fc, retaining the β - α - α - β structure described above. Because the IR is expressed in a eukaryotic system, disulfide bond

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formation and glycosylation patterns should mimic the wild-type receptor. The details of this recombinant protein construct are described in Bass *et al.* (1996).

In panning with the peptide library, the IR was immobilized directly onto a protein-binding plastic surface, and four rounds of panning and enrichment were carried out. Analysis of phage clones from rounds three and four showed that 114 of the 216 clones from the 20mer random peptide library and 17 of the 120 from the 40mer random peptide library bound to IR (Figures 1A, 1B, 2A, 2C, 4A, 6A, 10A and 10B). Of those clones tested competitively against insulin for receptor binding, all were blocked by the presence of natural ligand. This result indicated that these phage clones and insulin bind to the same site (or at least overlapping sites) on IR.

Sequence analysis of several clones shows that there are several distinct populations, designated as Groups 1 through 8 (Figures 1-8) (Figures 47 and 48). Several of the peptides based on the sequences for these groups have been chemically synthesized for subsequent testing. Group 1 (Formula 1 motif) peptides contain the consensus sequence FYxWF, and are believed to be agonistic in cell-based assays. Group 2 (Formula 6 motif) is composed of two peptides having a consensus sequence VYGR and two cysteine residues each. Thus, Group 2 peptides are capable of forming a cyclic peptide bridged with a disulfide bond. Group 3 (Formula 2 motif) peptides comprise the preferred consensus sequence F-Y-x-A/G-L/I-x-x-L (A/G denotes the alanine or glycine residue, and L/I denotes the leucine or isoleucine residue). Certain Group 3 peptides exhibit agonistic activity in cell-based assays (Figure 49). Neither of these consensus sequences have any significant linear sequence similarities greater than 2 or 3 amino acids with mature insulin.

Group 7 (Formula 4 motif) is composed of two exemplary peptides which do not have any significant sequence homology, but have two cysteine residues 13-14 residues apart, thus being capable of forming a cyclic peptide with a long loop anchored by a disulfide bridge.

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Example 10: ELISA Analyses of Phage

This series of experiments was designed to help characterize the different groups of consensus sequences found during the biopanning of IR. Phage were prepared from each group (two unique sequences each were attempted). Each phage was bound to insulin receptor and competition experiments were performed.

Phage Production. Each phage culture was started by the addition of 30 μ I of the master stock to 20 ml 2xYT-AG in 50 ml centrifugation tubes. Cultures were incubated at 37°C until OD₆₀₀ ~0.6-1.0. M13K07 helper phage were added to a concentration of ~5 x 10^{10} cfu ml⁻¹ and incubated at RT for 30 min. The cultures were centrifuged at ~2500g and 4°C for 20 min. The bacterial pellet was resuspended in 30 ml 2xYT-AK. The culture was transferred into 250 ml bottles and incubated O/N at 37°C. The culture was centrifuged at ~2500g and 4°C for 20 min (in 50 ml centrifuge tubes). The supernatant was transferred to new 50 ml centrifuge tubes.

Phage ELISA. Each well of the Nunc-ImmunoTM plates with the MaxiSorpTM surface were coated with either 50 μl of 2 ng/μl either IR or sIGF-1R in PBS overnight at 4°C. The wells were blocked with 200 μl of MPBS for 1.5 h at RT. Phage were added at 100 μl per well. Peptides were added as noted below and allowed to incubate at RT for 3 h. The plates were washed 3 times with PBST. A solution of 1:3000 diluted HRP:Anti-M13 conjugate at 100 μl per well of was added for 1 h. Following a repeat of the washing, 100 μl of ABTS was added for 15-30 min. The OD was measured using a SpectraMax 340 Microplate Spectrophotometer (Molecular Devices) at 405 nm.

Peptide Competition. Competition of phage displayed peptides by the addition of soluble peptides was carried out using the phage ELISA as described above. Twenty microliters of the stock synthetic-peptide solution was added to Row A. A series of 20 μl into 100 μl dilutions were performed until Row G. Twenty microliters were discarded from Row G to maintain 100 μl per well. Row H was reserved as no peptide wells. The starting

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concentration of the B6 peptide was 68 μ M for both receptors. For IR, the starting concentration for the C1 peptide was 48.5 μ M. Only 2 μ l of the C1 peptide were added to Row A of wells containing IGF-1R. Therefore, the starting concentration was 4.9 μ M. The volume was maintained by the addition of 18 μ l of the phage solution to Row A.

Natural Ligand Competition. The "Phage First" experiments were performed by adding 10 μ I of 5.5 μ M, 550 nM, or 55 nM insulin or IGF-1 in PBS to phage-containing wells in the phage ELISA. The working concentrations were 500 nM, 50 nM, and 5 nM. The volume of no ligand wells was maintained by the addition of 10 μ I PBS.

The "Ligand First" experiments were performed by added 50 μ l of 2 μ M, 200 nM, or 20 nM insulin or IGF-1 in PBS containing 0.5% Tween-20 to non-phage containing wells and allowed to incubate 15 min. Fifty microliters of the phage solution was then added to the wells and mixed well. The mixture was allowed to incubate for 2 h at RT and continue with the phage ELISA.

The data are provided in Table 7 and Figures 50A-50D. Sequences were confirmed on all clones by DNA sequencing.

TABLE 7: Phage Characterization Summary

	Absorbance Values			IR		sIGF-1R	
				Competitions		Competitions	
	NFM	sIGF-1	R IR	C1	B6	C1	В6
Group 1							
20D3	0.09	2.26	1.29	Υ	Υ	-	
B8	0.10	2.55	1.30	Υ	Y		
Group 2							
20A4	0.15	0.21	1.61	N	N	-	- '
D8	0.09	2.19	1.42	N	N	Υ	Υ
Group 3							
20E2	0.11	2.15	1.01	Υ	<u>Y</u>	-	
Group 4							
D10	0.12	0.14	0.73	N*	N	-	-
A2	1.35	2.00	1.79	N_	N	N_	
Group 5				İ			
D9-2	1.02	2.53	1.64	N	N	-	-
H4	1.16	1.14	1.41	N*	N	-	_
Group 6							
E8	0.10	2.00	1.34	Y	Y	-	
F2	0.09	2.08	1.43	Υ	Y	ļ -	
Group 7							V
F8	0.14	2.06	1.49	N	N	Y	Υ
Group 8	0.50	0.55	4.00	1	V		
40A2	0.56	0.55	1.90	Y*	Y	-	-
40H4	0.75	0.83	0.84	1 -	-	<u> </u>	

NFM = Non-fat milk

5 C1 peptide (D112) has the FYX₃WF Formula 1 motif and an amino acid sequence of DYKDCWARPCGDAANFYDWFVQQASKK

B6 peptide has the $FYX_8X_9LX_{11}X_{12}L$ Formula 2 motif and an amino acid sequence of WNTVDPFYHKLSELLREKK

Observations and Conclusions

- 1. The C1 and B6 peptides bind to IR. The C1 and B6 peptides expressed as phage-displayed peptides are negatively charged.
- 2. Groups 1, 3, and 6 (Formulas 1, 2 and 10, respectively), appear to be inhibited by both the C1 and B6 peptides when binding to IR and IGF-1R. All three groups behave with similar characteristics and similar affinities. They all bind to a common site, (Site 1) as shown by competition data.
 - 3. Group 2 (Formula 6 motif) phage clones have different properties despite their sequence similarity. The phage 20A4 is an IR-specific clone.
- 10 Its binding to IR is not inhibited by C1 or B6 peptides and therefore binds to Site 2. The phage D8 binds to both IR and IGF-1R. Inhibition by C1 peptide and B6 peptide occurs only when binding to IGF-1R. D8 is more sensitive to C1 and B6 peptide inhibition than Group 1, 3, and 6, suggesting an allosteric competition.
- 4. Some phage appear to have a plastic-binding component (binding to the wells of microtiter plates) in their sequences when high amounts of phage are used. The phage A2, D9-2, H4, 40F10, 40A2, and 40H4 have a significant background to their signals. With the exception of 40H4, all signals increase over this background signal in the presence of IR. The signals for phage A2 and D9-2 also increase over background for IGF-1R. It should be noted the phage for the IGF-1R binder B6 shows this similar characteristic.
 - 5. The Group 2 phage 20A4 and Group 4 phage D10 are specific for IR
 there is no detectable binding to IGF-1R. D10 may be inhibited by C1 peptide to a small extent.
 - 6. The phage for Group 7, F8 (Formula 4 motif) has characteristics similar to Group 2, D8 (Formula 6 motif). This clone binds to both IR and IGF-1R, but the C1 and B6 peptides only affect D8 binding when bound to IGF-1R. F8 is more sensitive to C1 and B6 peptide inhibition than Group 1,
- 30 3, and 6, (Formula 1, 2 and 10 motifs, respectively).

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Example 11: Cross-Reactivity Studies

Phage ELISA experiments show that the IGF-1R peptides H2 and E4 have detectable binding to IR while expressed as a phage fusion. Other IGF-1R-specific peptides such A6, C1, B6, and JBA5 do not have detectable binding to IR when expressed as phage.

A. Experimental Procedures

Phage Production. Each phage culture was started by the addition of 40 μ I of the MASTER stock to 20 ml 2xYT-AG in 50 ml centrifugation tubes. Cultures were incubated at 37°C until OD₆₀₀ ~0.6-1.0. M13K07 helper phage were added to a concentration of ~5 x 10¹⁰ cfu/ml and incubated at RT for 30 min. The cultures were centrifuged at ~2500g and 4°C for 20 min. The bacterial pellet was resuspended in 20 ml 2xYT-AK and incubated O/N at 37°C. The culture was centrifuged at ~2500 x g and 4°C for 20 min. The supernatant was transferred to new 50 ml centrifuge tubes

Phage ELISA. Each well of the Nunc-ImmunoTM plates with the MaxiSorpTM surface were coated with 50 μl of 2 ng/μl either IR or IGF-1R in PBS O/N at 4°C. The wells were blocked with 200 μl of 2% (w/v) Carnation non-fat dry milk in PBS for 1.5 h at RT. Phage were added at 100 μl per well. Peptides were added as noted below and allowed to incubate at RT for 3 h. The plates were washed 3X with PBST. A solution of 1:3000 diluted HRP:Anti-M13 Conjugate at 100 μl per well of was added for 1 h. Following a repeat of the washing, 100 μl of ABTS was added for 15-30 min. The OD₄₀₅ was measured using a SpectraMax 340 Microplate Spectrophotometer.

Peptide Competition. Peptide Competition Curves were produced during the phage ELISA across rows in triplicate. The stock synthetic peptide solution was added to Column 12 so that the total volume totaled 150 μl (additional phage solution was added when necessary). A serial dilution was made by transferring 50 μl from Column 12 into 100 μl in Column 11, 50 μl from Column 11 into 100 μl in Column 10, and continuing

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the serial dilution until Column 2. Fifty microliters were discarded from Column 2 to maintain 100 μ l per well. Column 1 was reserved as no peptide wells. The starting working concentrations for each peptide was: H2 – 50 μ M; H2C - 100 μ M; C1C - 100 μ M; D2C – 100 μ M; E4 – 33.3 μ M; C1 – 50 μ M; A6 - 100 μ M; and p53 - 100 μ M.

B. IGF-1R Peptide Competition

An experiment was designed to ascertain whether IGF-1R peptides have the ability to compete phage that bind to IR. Competition will occur in either IR- or IGF-1R-coated wells. The IGF-1R peptides H2, H2C, C1C, D2C, E4, C1, and A6 were tested for competition with two separate phage. The first, 20D3, (Figures 51A, 51C) is a phage discovered during panning of IR, but is also positive for binding to IGF-1R. The second, H2, (Figures 51B, 51D) is a phage found during panning of the IGF-1R, but is also positive for binding to IR. A p53-like peptide that binds to MDM2 was used as a negative control.

The Hill Plot data are provided in Table 8 below, and presented graphically in Figures 52A-52D.

IGF Receptor Insulin Receptor H2 Phage 20D3 Phage H2 Phage 20D3 Phage K_d r2 n Kd K_d Pept. n K_d n 762 0.981 H2 1.29 0 991 1.21 9812 0 979 1.07 1133 0.978 071 4958 480 0 926 H₂C 5055 0.975 1 02 3720 0.987 1 03 564 0 976 0.62 0.81 0 922 0 988 0 96 40 0 976 0.83 324 0 999 0.46 132 C1 1 37 19 CIC 0 945 0.70 1190 0.988 0 53 532 0.956 1.32 13475 0 990 1.00 34198 0.81 2491 0.995 0 96 2964 0 983 D2C 1 50 12454 0.995 1.34 33124 0.999 0 961 0.79 1435 0 979 0.71 387 0 994 6522 0.995 1.11 5868 E4 1.53

TABLE 8: Hill Plot Data

20 C. Observations and Conclusions

a. These peptides can bind to IR and inhibit binding of phage found by either panning IR (20D3) or IGF-1R (H2). This crossover event

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between the two receptors occurs despite negative results of many of these same phage-displayed peptides.

- b. Although the C1 peptide is the most potent inhibitor of phage binding, C1 peptide loses much of its potency advantage over the other peptides binding IR instead of IGF-1R. In addition, A6 gains potency when binding to IR relative to the other peptides. Combined, this suggests that the adjacent surfaces to this active site of the receptors are sufficiently different that peptides and small organic molecules specific for either receptor can be found.
- c. The Hill Coefficient of the peptides binding to IGF-1R is always1.5 to 2-fold higher than the same phage and peptide binding to IR.

Example 12: Competition of Phage Binding with Insulin

Many different peptides isolated from the random peptide libraries were tested for the ability to compete the natural ligand insulin. Clones tested were B8 (D103) (Formula motif 1), F4 (Formula motif 1), A7 (D122) (20A4) (Formula motif 6), D8 (D123; data not shown) (Formula motif 6), C6 (Formula motif 2), E8 (Formula motif 10), H4 (group 5; data not shown), A4 (group 6), G8 (group 7), G7 (Fc binder). H4 most likely binds non-specifically to the material from which the microtiter plate is made.

20 A. Insulin Competition Procedure

Receptors were coated at 100 μ g/ml, 50 μ l/well. After blocking with MPBS and washing 3x with PBST, insulin was added in the presence of 0.1% Tween-20 at 2 μ M, 100 nM, and 5 nM for 15 min before the addition of IR binding phage. The final concentration of insulin was 1 μ M, 50 nM and 2.5 nM. Reaction was incubated at RT for 1 h and wells were washed 3x with PBST (PBS with 0.05% Tween-20). Anti-M13 HRP was added and incubated for 1 h at RT. Wells were washed 3x with PBST before the addition of ABTS. Plates were read at 405 nm.

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B. Results

At high insulin dosage, all clones, except F4, G7, and H4 # (not shown), were inhibited; B8 showed the best inhibition, >50%. The apparent lack of binding of F4 (group 1) might be due to the insufficient level of phage present. G7 is a Fc binding phage is should not by inhibited by insulin. H4 is suspected to be a plastic-binding phage. The results are presented in Figure 54.

C. Conclusions

Insulin competition with a representative member from each group indicated that almost all of the groups competed with insulin; only the "plastic binders" and Fc binding phage did not compete. Different degrees of inhibition by these peptides (phage) imply that the peptides recognize different epitopes on or in the close proximity of the receptor active site.

Example 13: Synthetic Peptide 20A4 Competition Results

This experiment was performed as in Example 12. The 20A4 peptide (D122) starting concentration was 58 µM.

Results. The results are included in Table 7. The peptide 20A4 (D122) (A7) competes with Group 2 members (Formula 6 motif), Group 4 member (miscellaneous) D10, and Group 7 member (Formula 4 motif) F8 (D124). There is a partial inhibition of Group 6 member F2. The data is consistent with the conclusion that the site for 20A4 binding is different from the site for Group 1, Group 3, and Group 6.

Example 14: Peptide Fusions to the Maltose Binding Protein - Construction, Purification and Characterization of the Binding to the Insulin Receptor

A. Cloning

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The transfer of interesting peptide sequences from phage display to display as maltose binding protein (MBP) -fusions is desirable for several reasons. First, to obtain a more sensitive affinity estimate, the polyvalency of phage display peptides should be converted to a monovalent system. For this purpose, the peptide sequences are fused to MBP that generally exists as a monomer with no cysteine residues. Second, competition experiments can be carried out with the same or different peptides, one phage displayed and the other fused to MBP. Lastly, purified peptides can be obtained by cleavage of the fusion protein at a site engineered in the DNA sequence.

Figure 55 shows a schematic drawing of the MBP-peptide construct. In the construct, the N-terminus of the peptide sequence is fused to the Cterminus of the MBP. Two peptide-flanking epitope tags are included, a shortened-FLAG at the N-terminus and E-Tag at the C-terminus. The corresponding gene fusion was generated by ligating a vector fragment encoding the MBP in frame with a PCR product encoding the peptide of interest. The vector fragment was obtained by digesting the plasmid pMALc2 (New England Biolabs) with EcoRI and HindIII and then treating the fragment with shrimp alkaline phosphatase (SAP; Amersham). The digested DNA fragment was resolved on a 1% agarose gel, excised, and purified by QIAEXII (QIAGEN). The 20-amino acid peptide sequences of interest were initially encoded in the phage display vector pCANTAB5E (Pharmacia). To obtain these sequences, primers were synthesized which anneal to sequences encoding the shortened FLAG or E-Tag epitopes and also contain the required restriction enzyme sites EcoRI and HindIII. PCR products were obtained from individual phage clones and digested with restriction enzymes to yield the insert fragment. The vector and insert were ligated overnight at 15°C. The ligation product was purified using QIAquick

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spin columns (QIAGEN) and electroporations were performed at 1500 v in an electroporation cuvette (0.1 mm gap; 0.5 ml volume) containing 10 ng of DNA and 40 µl of *E. coli* strain ER2508 (RR1 *lon:mini*Tn10(Tet^r) (*malB*) (*argF-lac*)*U169* Pro⁺ *zjc*::Tn5(Kan^r) *fhuA2*) electrocompetent cells (New England Biolabs). Immediately after the pulse, 1 ml of pre-warmed (40°C) 2xYT medium containing 2 % glucose (2xYT-G) was added and the transformants were grown at 37°C for 1 h. Cell transformants were plated onto 2xYT-AG plates and grown overnight at 37°C. Sequencing confirmed the clones contained the correct constructs.

10 B. Small-Scale Expression of Soluble MBP-Peptide Fusion Proteins

E. coli ER2508 (New England Biolabs) carrying the plasmids encoding MBP-peptide fusion proteins were grown in 2xYT-AG at 37°C overnight (250 rpm). The following day the cultures were used to inoculate media (2x YT containing-G) to achieve an OD₆₀₀ of 0.1. When the cultures reached an OD₆₀₀ of 0.6, expression was induced by the addition of IPTG to a final concentration of 0.3 mM and then cells were grown for 3 h. The cells were pelleted by centrifugation and samples from total cells were analyzed by SDS-PAGE electrophoresis. The production of the correct molecular weight fusion proteins was confirmed by Western blot analysis using the monoclonal antibody anti-E-Tag-HRP conjugate (Pharmacia).

C. Large-Scale Expression of Soluble MBP-Peptide Fusion Proteins

E. coli ER2508 carrying plasmids encoding the MBP-peptide fusion proteins were grown in 2xYT-AG media for 8 h (250 rpm, 37°C). The cultures were subcultured in 2xYT-AG to achieve an OD₆₀₀ of 0.1 and grown at 30°C overnight. This culture was used to inoculate a fermentor with medium of following composition (g/l):

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	Glucose	3.00
	$(NH_4)_2SO_4$	5.00
	MgSO ₄ •7H2O	0.25
	KH₂PO₄	3.00
5	Citric Acid	3.00
	Peptone	10.00
	Yeast extract	5.00
	pH 6.8	

The culture was grown at 700 rpm, 37° C until the glucose from the medium was consumed ($OD_{600} = \sim 6.0 - 7.0$). Expression of the fusion protein was induced by the addition of 0.3 mM IPTG and the culture was grown for 2 h in fed-batch mode fermentation with feeding by 50 % glucose at a constant rate of 2 g/l/h. The cells were removed from the medium by centrifugation. Samples of the cell pellet were analyzed by SDS-PAGE followed by the Western blot analysis using the mouse monoclonal antibody anti-E-Tag-HRP conjugate (Pharmacia) to visualize the expressed product.

D. Purification

The cell pellets were disrupted mechanically by sonication or chemically by treatment with the mild detergent Triton X-100. After removal of cell debris by centrifugation, the soluble proteins were prepared for chromatographic purification by dilution or dialysis into the appropriate starting buffer. The MBP fusions were initially purified either by amylose affinity chromatography or by anion exchange chromatography. Final purification was performed using anti-E-Tag antibody affinity columns (Pharmacia). The affinity resin was equilibrated in TBS (0.025 M Trisbuffered saline, pH 7.4) and the bound protein was eluted with Elution buffer (100 mM glycine, pH 3.0). The purified proteins were analyzed for purity and integrity by SDS-PAGE and Western blot analysis according to standard protocols.

For BIAcore analysis of fusion protein and synthetic peptide binding to insulin receptor, insulin (50 μ g/ml in 10 mM sodium acetate buffer pH 5) was immobilized on the CM5 sensor chip (Flowcell-2) by amine coupling.

Flowcell-1 was used for background binding to correct for any non-specific binding. Insulin receptor (450 nM) was injected into the flow cell and the binding of IR to insulin was measured in resonance units (RUs). Receptor bound to insulin gave a reading of 220 RU. The surface was regenerated with 25 mM NaOH. Pre-incubation of receptor with insulin in a tube at RT completely abrogated the response units to 16 RU. Thus, the system was validated for competition studies. Several maltose-binding fusion proteins, peptides and rVabs were pre-incubated with insulin receptor before injecting over the insulin chip for competition studies. The decrease in binding/resonance units indicates that several MBP-fusion proteins can block the insulin binding site. The results are shown in Tables 9 and 10. The amino acid sequences referred to in the tables are identified in Figures 47 and 48, except the 447 and 2A9 sequences, which are shown below.

15 **TABLE 9:** BlAcore Results—Fusion Proteins Compete for Binding to IR

	Incubation Mixtures	Result (RUs)	Sequence Type	
Controls	Insulin Receptor (IR) 450 nM	220	Positive Control	
	Insulin (8.7 μM)	16	Negative Control	
MBP Fus. Prots.	A7 (20A4)-MBP (4.1 μM) + IR	43	Formula 6 Motif	
	D8-MBP (1.6 µM) + IR	56	Formula 6 Motif	
	D10-MBP (3.4 µM) + IR	81	Formula 11 Motif	
	447-MBP (11.5 μM) + IR	195	hGH Pept. Fus.	
	MBP (13 μM) + IR	209	Negative Control	

TABLE 10: BlAcore Results—Synthetic peptides compete for binding to IR

Incubation Mix	% Binding	Result (RUs)	Sequence Type
IR	100	128	Positive control
IR + 20D1	41	51.8	Formula 1 Motif
IR +D8	33	41.6	Formula 6 Motif
IR + 20C11	38	49	Formula 2 Motif (bkg high)
IR + H2	27	34.6	IGF (phosphorylated band)
IR + 2A9	100	128	IGF(bkg high)
IR + 20A4	33	41.8	Formula 6 Motif
IR + p53wt	97	124.5	P53 wild type

The concentration of each peptide was about 40 µM and the concentration of IR was 450 nM. The 447 peptide sequence is: LCQRLGVGWPGWLSGWCA. The 2A9 peptide sequence is: LCQSWGVRIGWLTGLCP.

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Example 15: Insulin Receptor Competition ELISA Using Phage Displayed Peptides and MBP-Peptide Fusion Proteins

To determine whether the binding sites (contact sites) on the insulin receptor for the various peptides are similar, the purified fusion proteins were used in ELISA competition experiments with phage displayed peptides from various groups. Phage-displayed peptides, which were able to bind to IR, were classified into various groups according to consensus sequences identified (see Figures 47 and 48). Peptide sequences of interest were fused to the C-terminus of MBP as previously described. The protein fusion constructs were expressed as soluble proteins, purified, and the protein concentrations were determined. The purified fusion proteins were used in ELISA competition experiments with phage displayed peptides from the various groups as shown in Table 11.

As expected, the fusion proteins containing A7 (20A4), D8, D10, and F8 peptides were able to compete the corresponding identical peptide sequence displayed on phage in the range of 28-54% of the control value. The fusion protein, MBP-A7, was able to significantly compete (<54%) phage-displayed peptides D8, D10, and F8. The other fusion protein from Group 2 (Formula 6 motif), MBP-D8, was able to compete A7 and D10 peptides displayed on phage. Furthermore, the Group 7 (Formula 4 motif) fusion protein MBP-F8 competed A7 and D10 phage displayed peptides. Figures 56A and 56B show the plotted data from Table 11. In Figure 56A, a clear pattern is seen where significant (≤ 54%) competition reactions occur between fusion proteins and phage-displayed peptides which have in common the presence of at least two cysteine residues (see Figures 47 and 48 for peptide sequences).

Also striking is the observation that the cysteine containing fusion proteins were not able to compete phage displayed peptides from Group 1 (Formula 1 motif), which contain the consensus (IGF A6-like) sequences and are without cysteine residues (Figure 56A). In Figure 56B, the fusion proteins containing the Group 1 (Formula 1 motif) consensus sequences

were not able to compete to a significant extent any of the phage-displayed peptides from any of the groups. It should be noted that the corresponding identical phage from Group 1 was not tested. The data support the conclusion that the cysteine-containing peptides bind to a contact site (Site 2) which is different than the contact site (Site 1) required for the consensus containing peptides (Group 1, (Formula 1 motif)) to bind the insulin receptor.

TABLE 11

Phage Displayed		Grou	Group 1		up 2	Group 4	Group 7	Control
Donti	doc	MBP-E7	MBP-H8	MBP-A7	MBP-D8	MBP-D10	MBP-F8	MBP-447
Pepti	aes	1.6 µM	1.6 µM	(20A4)	2 μΜ	4 μM	2.8 µM	14 µM
				5 µM				
Group 1	B8	265	264	329	267	274	240	299
	20 D3	196	196	250	170	218	208	186
Group 2	D8	138	135	<u>53</u>	<u>54</u>	129	111	160
	A7	133	103	<u>28</u>	<u>54</u>	125	<u>21</u>	116
,	(20A4)							
Group 3	20 E2	80	106	100	69	84	161	100
Group 4	A2	92	92	88	74	105	98	79
	D10	92	60	20	<u>20</u>	<u>36</u>	20	117
Group 6	F2	91	97	88	83	92	83	101
	E8	86	75	164	99	94	86	110
Group 7	F8	99	93	44	63	82	<u>43</u>	138
Group 8	40 A2	80	74	118	84	95	80	90

Data reported in the table above was obtained as follows: IR was

coated on a 96-well plate with 50 µl of 2 ng/µl IR and incubated overnight at

4°C. The wells were then blocked with MPBS for 1 h. The fusion proteins

(mixed #1:5 with MPBS) were added to the wells and incubated at RT for 30

min. An equal volume of phage (displaying various peptides from each of
the groups) was then added and incubated for 1.5 h. The control well

contained only phage and an equal volume of buffer. The plate was washed
3 times in PBST and then incubated with HRP/anti-M13 conjugate for 45

min. The plate was washed again and then the ABTS substrate added. The

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values indicate readings taken at OD_{405} which were normalized as percent control. The control fusion protein MBP-447 contains a peptide that binds the growth hormone receptor. Peptides in bold type contain cysteine residues. Underlined and in bold are values which are $\leq 54\%$ of control values.

Example 16: Biopanning the rVab Library

The same rVab library described in Example 5 and panned for members that bound IGF-1R was also panned for members that bind IR. Human insulin receptor was diluted to 1 mg/ml in 50 mM sodium carbonate buffer, pH 9.5. One hundred microliters of this solution was added to an appropriate number of wells in a 96-well microtiter plate (MaxiSorp plates, Nunc) and incubated overnight at 4°C. The wells were then blocked by adding 100 µl of MPBS to each well and incubating at RT for 1 h.

The phage were incubated with MPBS for 30 min at RT, then 100 µl of the phage solution were added to each well and incubated for 2 h at RT. In the first round, the input phage titer was about 10¹³ cfu/ml. The input phage titer was about 10¹¹ cfu/ml in subsequent rounds.

The wells were washed 13 times with 200 μ I/well of MPBS, then washed once with PBS (200 μ I/well). The bound phage were eluted by adding to each well 100 μ I of 20 mM glycine-HCI, pH 2.2. After 30 s, the phage was transferred to an Eppendorf tube and the solution was neutralized by adding 50 μ I of 1 M Tris-HCI, pH 8.0, per volume from each well.

TG1 cells were grown to the mid-log phase ($OD_{600} = 0.5$). Equal volumes of the TG1 cell culture and the neutralized phage solution were mixed together, incubated for 1 h at 37°C without shaking, and then plated onto two 24 cm x 24 cm 2xYT-AG agar plates. The next morning, cells were removed by scraping the surface of the agar plates, and were then suspended in 24 ml 2xYT and stored in 10% glycerol at -80°C.

The input phage for the subsequent rounds of biopanning was prepared by growing 100 µl of the cells from these frozen stocks, followed

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by phage preparation according to the Protocol Preparation of Phage described below.

Protocol: Preparation of Phage

The general protocol for phage preparations used to prepare phage 5 displayed rVabs is described below.

- 1. Phagemid-containing TG1 cells were grown to $OD_{600} = 0.5$ in 2xYT-AG media at 37°C with shaking (250 rpm).
- 2. M13K07 helper phage were then added (at MOI = 20), and the cells were incubated for 1 h at 37° C with gentle shaking (150 rpm).
- 3. Following infection, cells were pelleted by centrifugation at 1,000 g for 20 min and the supernatant containing the helper phage were discarded.
 - 4. The cell pellet was resuspended in the initial culture volume in 2xYT-AK and grown overnight at 30°C with shaking (250 rpm).
 - 5. The cells from the overnight culture were pelleted at 3,000 g for 30 min at 4°C and the supernatant containing the phage was recovered.
 - 6. The supernatant was adjusted to contain 4% PEG, 500 mM NaCl and chilled on ice for 1 h. The precipitated phage was pelleted by centrifugation at 10,000 x g for 30 min. The pellet was resuspended in MPBS.

Example 17: Expression and Characterization of IR Binding rVab Clones

A. Infection of E. coli HB2151 Cells

a. To prepare the log-phase cells, 2xYT media was inoculated with *E. coli* strain HB2151 cells (genotype) from a fresh minimal medium plate, and the cells were grown to $OD_{600} = 0.5$ at 37° C with shaking (250 rpm).

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- b. Fifty microliters of the pool phage from biopanning round 3 (or round 4) were transferred to 2 ml of the log phase HB2151 cells. The cells were incubated with gentle shaking for 1 h at 37°C.
- c. The cells were diluted appropriately with the 2xYT media, plated on 2xYT-AGN plates and incubated overnight at 30°C.

B. Preparation of Soluble Antibodies for Screening IGF Repetition

- a. Four hundred microliters of 2xYT-AG media were added to each cluster tube (in a rack of 96 tubes in a microtiter format, Costar #4411).
- b. The media in cluster tubes were inoculated by transferring the individual well-isolated colonies from the 2xYT-AGN plates using sterile toothpicks; the cluster tubes were then incubated overnight at 30°C with shaking (250 rpm). The array of bacterial cultures in cluster tubes constitutes the Master Plate.
- c. The next day, the Master Plate was duplicated by transferring 40 μ l of the saturated culture from each tube of the Master Plate to 400 μ l of 2xYT-AG medium in a new set of cluster tubes. The new array of duplicated cultures in the microtiter plate format was labeled S1.
- d. Plate S1 was incubated for 2 h at 30°C with shaking (250 rpm), and then centrifuged at 1,000 X g for 20 min at RT in a centrifuge equipped with microtiter plate adapters.
- e. The supernatant was carefully removed from each cluster tube and discarded to an appropriate waste container. Four hundred microliters of the 2xYT-Al medium (no glucose added) was added to each tube in plate S1, and the plate was incubated overnight at 30°C with shaking (250 rpm).
- f. Plate S1 was centrifuged as described above, and 320 μl of each supernatant (containing the soluble recombinant antibodies) was carefully transferred to a corresponding tube in a new set of 96 cluster tubes. The new plate was labeled S2.

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g. Eighty microliters of the MPBS blocking buffer was added to each tube of plate S2 (already containing 320 µl of the supernatant) and incubated for 10 min at RT. This rVab preparation was now ready to be used in an ELISA performed described above.

C. Detection of rVab Binding Using HRP/Anti-E-Tag Conjugate

- a. A microtiter plate was coated with the target protein and blocked as previously described. Some of the wells of the microtiter plate were coated with an unrelated antigen to serve as a negative control.
- 10 b. The rVab preparation prepared above was diluted two-fold with the MPBS blocking buffer. Two hundred microliters of this solution was added to a set of antigen-coated and control wells.
 - c. The plate was incubated for 2 h at RT, and then washed 3 times with PBST.
 - d. The HRP/Anti-E-Tag conjugate was diluted 1:4,000 in the MPBS blocking buffer. Two hundred microliters of the diluted conjugate was added to each well, and the plate was incubated for 1 h at RT.
 - e. The microtiter plate was washed 3 times with PBST.
- f. Two hundred microliters of the ABTS solution was added to each well, the microtiter plate was incubated for 20 min at RT, and the absorbance of each well was read at 405 nm in an appropriate microtiter plate reader.

D. Production of Soluble rVabs

- a. A suitable rVab clone in HB2151 cells was transferred from a
 25 2xYT plate to 3 ml of 2xYT-AG media, and the culture was incubated overnight at 30°C with shaking (250 rpm).
 - b. Part of the overnight culture (2.5 ml) was added to 25 ml of the 2xYT media and incubated for 1 h at 30°C with shaking (250 rpm).
- c. The culture was centrifuged at 1000 g for 20 min at RT, and the supernatant was removed from the pelleted cells and discarded. The

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pelleted cells were resuspended in 25 ml of 2xYT-Al media (no glucose is added) and were incubated overnight at 30°C with shaking (250 rpm).

E. Purification of rVabs

The Pharmacia RPSA Purification Module kit was used (Cat. #17-1362-01), and purification was performed according to the manufacturer's directions.

- a. A syringe was filled with the Elution Buffer (100 mM glycine, pH 3.0).
- b. The stopper on the top of the anti-E-Tag column was removed
 and a drop of the Elution Buffer was added to the top of the column. The syringe was connected to the column with the Luer adapter. The connection was "drop to drop" to avoid introducing air into the column.
 - c. The twist-off end was removed and the column was washed with 15 ml of the Elution Buffer at a flow rate of 5 ml/min, followed immediately by 25 ml Binding Buffer (10X Binding Buffer: 0.20 M Phosphate Buffer, 0.05% NaN₃, pH 7.0).
 - d. Sample was applied with a peristaltic pump P-1 (Pharmacia, Cat. #19-4611-02) at a flow rate of 5 ml/min at 4°C.
 - e. The column was washed with 25 ml of the Binding Buffer at a flow rate of 5 ml/min to remove unbound *E. coli* proteins.
 - f. Bound rVab was eluted from the anti-E-Tag column with the Elution Buffer. The first 4.5 ml of material eluted from the column was discarded.
- g. The next 5 ml (containing the purified E-tagged rVab) wascollected in either one or several fractions.
 - h. The column was immediately re-equilibrate with 25 ml of the Binding Buffer for use with the next sample.

Example 18: Competition ELISA with rVabs

For IC₅₀ determinations, microtiter plates were coated with IR and blocked as in Example 9. Soluble rVabs were prepared as described in

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Example 9. Prior to addition of soluble rVabs to the plates, 100 l/well of 100 nM insulin solution in PBS was added to duplicate wells. After incubation for 1 h at RT, the prepared soluble rVabs were added to each well (100 µl/well) without removing the insulin solution. After incubation for 1 h at RT, the wells were washed and the color was developed as described in Example 9.

Example 19: Activities of rVabs in the Cell-Based Assay

Agonistic and antagonistic activities of IR-specific soluble rVabs were tested in 969 cells stably transfected with the gene encoding the human IR and IRS-1 (insulin receptor substrate). The resulting cell line requires IL-3, IL-4, or insulin for growth. Negative control cell lines do not require IRS-1 for growth. The cells were grown in RPMI 1640 media containing 10% FCS and 20 units of IL-3 per ml. Cells were seeded at 30,000 cells/well in 50 µl PRMI1640 (without IL-3) media containing horse serum instead of FCS to reduce the background. Fifty microliters of either insulin or soluble rVabs at different concentrations were added to duplicate wells, followed by incubation for 18 h in a CO₂ incubator. The cell proliferation assays were performed using WST-1 reagent. The WST-1 tetrazolium salt is cleaved to form formazan by the succinate-tetrazolium reductase system that is active only in viable cells. An increase in the number of cells results in an increase of the overall enzymatic activity of the dehydrogenase that results in a higher absorbance at 450 nm. Ten microliters of WST-1 reagent were added and the plate was incubated for 1-4 h at 36°C. Figure 60 shows the results of these studies. As can be seen, rVab 12h10 was able to induce an agonist response in 32D cells expressing IR with an ED₅₀ of approximately 50 nM.

Example 20: IR Activation Assays

The kinase receptor activation ELISA is a functional assay based on the ability of a sample to stimulate or inhibit autophosphorylation of the insulin receptor construct that has been transfected into 32D cells (Wang et

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al., 1993; clone 969). The assay procedure begins with the cell stimulation. The IR transfected 32D cells were seeded at 5 x 10⁶ cells/well in 96-well tissue culture plates and incubated overnight at 37°C. Samples were diluted 1:10 in the stimulation medium (PRIM1640 with 25 nM HEPES pH 7.2) plus or minus insulin. The culture media was decanted from the cell culture plates, and the diluted samples were added to the cells. The plates were incubated at 37°C for 30 min. The stimulation medium was decanted from the plates, and cell lysis buffer (50 mM HEPES pH 7.2, 150 mM NaCl, 0.5% Triton X-100, 1 mM AEBSF, 10 KIU/ml aprotinin, 50 μM leupeptin, and 2 mM sodium orthovanadate) was added. The cells were lysed for 30 min.

In the ELISA portion of the assay, the cell lysates were added to the BSA-blocked anti-IR unit mAb (Upstate Biotechnology, Lake Placid, NY) coated ELISA plates. After a 2 h incubation, the plates were washed 6 times with PBST and biotinylated anti-phosphotyrosine antibody (Upstate Biotechnology) is added. After another 2 h incubation, the plates were again washed 6 times. Streptavidin-Eu was then added, and the plates were incubated for 1 h. After washing the plates again, EG&G Wallac enhancement solution (100 mM acetone-potassium hydrogen pthalate, pH 3.2; 15 mM 2-naphtyltrifluoroacetate; 50 mM tri(n-octyl)-phosphine oxide; 0.1% Triton X-100) was added into each well, and the plates were placed onto a shaker for 20 min at RT. Fluorescence of samples in each well was measured at 615 nm using a VICTOR 1420 Multilabel Counter (EG&G Wallac).

Alternatively, IR autophosphorylation was determined using a holoenzyme phosphorylation assay. In accordance with this assay, 1 μ I of purified insulin receptor (isolated from a Wheat Germ Agglutinin Expression System) was incubated with 25 nM insulin, or 10 or 50 μ M peptide in 50 μ I autophosphorylation buffer (50 mM HEPES pH. 8.0, 150 mM NaCl, 0.025% Triton-X-100, 5 mM Mn₂Cl, 50 μ M sodium orthovanadate) containing 10 μ M ATP for 45 min at 22°C. The reaction was stopped by adding 50 μ I of geI loading buffer containing β -mercaptoethanol (Bio-Rad Laboratories, Inc.,

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Hercules, CA). The samples were run on 4-12% SDS-polyacrylamide gels. Western Blot analysis was performed by transferring the proteins onto nitrocellulose membrane. The membrane was blocked in PBS containing 3% milk overnight. The membrane was incubated with anti-phosphotyrosine 4G10 HRP labeled antibody (Upstate Biotechnology) for 2 h. Protein bands were visualized using SuperSignal West Dura Extended Duration Substrate Chemiluminescence Detection System (Pierce Chemical Co.).

Example 21: Development of IR Assays Using Soluble rVab Antibodies and Biotinylated Peptides

- Heterogeneous Time-Resolved Fluorescence Assay. Sixty a. microliters of insulin receptor (60 ng/well) was coated onto 96-well lowfluorescence MaxiSorp (Nunc) plates overnight at 4°C. The plates were blocked with TBS containing 2% milk and 0.5% BSA for 1 h at RT followed by three TBS washes. To test binding of peptides to insulin receptor, serial dilutions of biotinylated peptides were added to IR coated plates for 2 h to overnight. After TBS wash, europium-labeled streptavidin at 1 μg/ml in assay buffer (100 mM Tris-HCl, pH 7.8; 150 mM NaCl; 0.5% BSA; 0.05% bovine Ig; 0.05% NaN₃; 0.01% Tween-20) was added to the plates and incubated for 1 h. To test binding of rVab antibodies to IR, Eu-labeled rVab antibodies in assay buffer were added to the plates and incubated for 2 h to overnight. After incubation with Eu-labeled streptavidin (for peptide test) or europium-labeled rVabs, the plates were washed 5 times with Tris-buffered saline (pH 7.5) containing 0.1% Tween-20 (TTBS) and tapped dry. Sixty microliters of EG&G Wallac enhancement solution (100 mM acetonepotassium hydrogen pthalate, pH 3.2; 15 mM 2-naphtyltrifluoroacetate; 50 mM tri(n-octyl)-phosphine oxide; 0.1% Triton X-100) was added into each well, and the plates were placed onto a shaker for 20 min at RT. Fluorescence of samples in each well was measured at 615 nm using a VICTOR 1420 Multilabel Counter (EG &G Wallac).
- b. <u>Homogeneous Time-Resolved Fluorescence Assay.</u> A mixture of 27 nM Cy5-labeled rVab 43G7 and 6-8 nM LANCE-labeled IGF-1R

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(EG&G Wallac) in Tris-buffered saline containing 0.1% BSA is added to 96-or 384-well white low-fluorescence plates (Nunc) for 2 h or overnight. For library screening, 20 μ M of small organic molecules in 2 % DMSO are included in the mixture. Unlabeled rVab 43G7 at 50 nM or IGF at 3 μ M are used as positive controls. Fluorescence of samples in each well is measured at both 615 nm and 665 nm using a VICTOR 1420 Multilabel Counter (EG &G Wallac).

Example 22: Binding of Synthetic Peptides to Insulin Receptor

A series of synthetic peptides were synthesized and biotinylated (Anaspec, Inc., San Jose, CA). The binding affinities of these peptides to IR and IGF-1R were tested. Most of these peptides bind to IR at micromolar range (Figure 63). Comparison of binding of biotinylated C1 peptide to IGF-1R and IR is shown in Figure 64, which indicates that binding of C1 to IGF-1R is at the nM range while binding to IR is at the micromolar range. A series of unlabeled peptides or soluble rVab were added to test competition binding to IR (Figure 65). H2C peptide at 30 μM appears to compete for binding to IR with biotinylated peptides from group 1 (Formula 1 motif) (20D1 and 20D3) and the two A6-based peptides (C1 and H2) but not compete with peptides from group 2 (Formula 6 motif) (20A4 and D8), group 3 (Formula 2 motif) (20C11) or the IGF peptide A9. The 33 F7 soluble rVab antibody competes with group 1 and 2 peptides as well as C1 peptide, however, it does not compete with 20C11 or 2A9. Figure 66 shows that H2C competition with biotinylated peptides, 20D3, H2, and C1, binding to IR is dose-dependent. C1C peptide also competes with C1 for IR binding (Figure 67).

Example 23: Competition for Binding to rVab 12H10 by Peptides and MBP-Peptide Fusion Proteins

Several peptides and four MBP-peptide fusion peptides were tested for competition of binding to IR with soluble rVab 12H10. Figure 68 shows

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that C1 and H2C at 30 μ M inhibit binding to 40-50% of control and C1C at 30 μ M inhibit to 60%. B6 and growth hormone do not compete with binding of 12H10 to IR. Four MBP-peptide fusion proteins (D10, 20A4, E7 and H8) all inhibit binding of 12H10 to IR to 20-30 % of control (Figure 69).

5 Example 24: Effects of Small Organic Molecules on IR Phosphorylation

Organic molecules positive for binding to IGF-1R and negative controls can be tested for their effects on phosphorylation of insulin receptor.

Example 25: Method for Determination of Insulin Receptor Binding of Peptides

In other insulin binding assays, IR was incubated with 125 I-labeled insulin at various concentrations of test substance and the K_d was calculated. According to this method, human insulin receptor (HIR) or human IGF-1 receptor (HIGF-1R) was purified from transfected cells after solubilization with Triton X-100. The assay buffer contains 100 mM HEPES (pH 7.8), 100 mM NaCl, 10 mM MSG, 0.5% human serum albumin, 0.2% gammaglobulin and 0.025% Triton X-100. The receptor concentration was chosen to give 30-60% binding of 2000 cpm (3 pM) of its 125 I-labeled ligand (TyrA14- 125 I-HI or Tyr31- 125 I-IGF1) and a dilution series of the substance to be tested was added. After equilibration for 2 days at 4°C, each sample (200 µI) was precipitated by addition of 400 µI 25% PEG 6000, centrifuged, washed with 1 mI 15% PEG 6000, and counted in a gamma-counter.

The insulin/IGF-1 competition curve was fitted to a one-site binding model and the calculated parameters for receptor concentration, insulin affinity, and non-specific binding were used in calculating the binding constants of the test substances. Representative curves for insulin and IGF-1 are shown in Figures 71A-71N.

The sequences of certain peptides analyzed are shown in Table 12, except for peptides D125 and D126. Synthetic peptides are numbered D1XX. D117K is an analog of D117 with an extra N-terminal lysine added

for facilitate solubility. Peptides produced recombinantly by phage are indicated as D1XXA.

The peptides are all biotinylated in the side chain of the C-terminal lysine (except D117A). The peptides produced recombinantly are C-terminal acids, whereas the synthetic peptides are C-terminal amides.

The results of the binding and phosphorylation assays are shown in Table 13.

TABLE 12

Name	Sequence	Motif
D101	KIGGQGQHQDGNFYDWFVEALAKK	1
D102	KVLQARHGCDSVSDCFYEWFAKK	1
D103	KWSALLSVMDTGFYAWFDDAVKK	1
D104	KGHSWALVRHVDRLFYEWFDLKK	1
D105	KRDKPTDQEEQNWSFYEWFRHKK	1
D106	KVFWNCRSQQLDFYEWFEQAAKK	1
D107	KLESHYVVPQAALDRLFYSWFSKK	1
D108	KFYGWFSRQLSLTPRDDWGLPKK	1
D109	KSAPGLVSNKQDGLFYSWFREKK	1.
D110	KRGGGTFYEWFESALRKHGAGKK	1
D111	KDPERMQSDVGFYEWFRAAVGKK	1
D112	DYKDCWARPCGDAANFYDWFVQQASKK	1
D113	DYKDVTFTSAVFHENFYDWFVRQVSKK	1
D114	SAKNFYDWFVKK	1
D115	ADKNFYDWFMAAKK	1
D116	DYKDLCQSWGVRIGWLAGLCPKK	9
D117	FHENFYDWFVRQVSKK	1
D117K	KFHENFYDWFVRQVSKK	1
D118	DYKDFYDAIDQLVRGSARAGGTRDKK	2
D119	KDRAFYNGLRDLVGAVYGAWDKK	2
D120	KVRGFQGGTVWPGYEWLRNAAKK	10
D121	KSMFVAGSDRWPGYGVLADWLKK	10
D122	KEIEAEWGRVRCLVYGRCVGGKK	10
D123	KWLDQEWAWVQCEVYGRGCPSKK	6
D124	KHLCVLEELFWGASLFGYCSGKK	4
D101A	KIGGQGQHQDGNFYDWFVEALAKK	1
D102A	KVLQARHGCDSVSDCFYEWFAKK	1
D112A	DYKDCWARPCGDAANFYDWFVQQASKK	1
D113A	DYKDVTFTSAVFHENFYDWFVRQVSKK	1
D117A	FHENFYDWFVRQVSKK	1
D119A	KDRAFYNGLRDLVGAVYGAWDKK	2
D122A	KEIEAEWGRVRCLVYGRCVGGKK	1.0
D123A	KWLDQEWAWVQCEVYGRGCPSKK	6
D124A	KHLCVLEELFWGASLFGYCSGKK	4

TABLE 13

Name	K _d (μM) HIR	K _d (μM) HIGF1R	Ratio	Autophosph. Blot
D101	0.51	13	25	-
D102	1.2	7.4	6.2	
D103	0.74	15	20	_
D104	20	>20		-
D105	2.8	12	4.3	-
D106	0.97	6.2	6.4	-
D107	1.1	9.7	8.8	+
D108	2.3	19	8.3	
D109	3.6	12	3.3	_
D110	0.84	1.4	1.7	_
D111	0.62	3.2	5.2	-
D112	0.49	0.05	0.1	_
D113	0.75	5.4	7.2	- (prec)
D114	8.1	>20	>2.5	0
D115	8.1	>20	>2.5	0
D116	4.4	8.1	1.8	0
D117	0.70	6.1	8.6	+
D117K	0.82	9.1	11.1	
D118	0.25	1.3	5.2	+
D119	4.5	13	2.9	+
D120	0.37	2.2	5.9	
D121	1.1	7.4	6.7	_
D122	1.2	>20	>17	0
D123	0.55	16	29	0
D124	0.04*	8.2	200	-
D101A	0.27	11.0	41	
D102A	0.97	16.0	16	
D112A	0.19	0.02*	0.1	
D113A				
D117A	0.60	5.1	8.5	
D119A	3.0	2.5	0.8	
D122A	1.0	>20	>20	
D123A	1.3	>20	>15	
D124A	0.09*	>20	>200	
D125A	2.6	>20	>8	
D126A	1.4	18	13	

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Example 26: Determination of Insulin Agonist Activity Based On ³H-Glucose Uptake into Adipocytes

Insulin increases uptake of ³H glucose into adipocytes and its conversion into lipid. Incorporation of ³H into the lipid phase was determined by partitioning of lipid phase into a scintillant mixture, which excludes water-soluble ³H products. The effect of compounds on the incorporation of ³H glucose at a sub-maximal insulin dose was determined, and the results expressed as increase relative to full insulin response. The method was adapted from Moody *et al.* (1974).

Mouse epididymal fat pads were dissected out, minced into degradation buffer (Krebs-Ringer 25 mM HEPES, 4% HSA, 1.1 mM glucose, 0.4 mg/ml Collagenase Type 1, pH 7.4), and degraded for up to 1.5 h at 36.5°C. After filtration, washing (Krebs-Ringer HEPES, 1% HSA) and resuspension in assay buffer (Krebs-Ringer HEPES, 1% HSA), cells were pipetted into 96-well Picoplates (Packard), containing test solution and approximately an ED_{20} insulin. The assay was started by addition of 3H glucose (Amersham TRK 239), in a final concentration of 0.45 mM glucose. The assay was incubated for 2 h, 36.5°C, in a Labshaker incubation tower, 400 rpm, then terminated by the addition of Permablend/Toluene scintillant (or equivalent), and the plates sealed, before standing for at least 1 h and detection in a Packard Top Counter or equivalent. A full insulin standard curve (8 dose) was run as control on each plate. Data are presented graphically, as effect of compound on an (approx) ED₂₀ insulin response, with data normalized to a full insulin response. The assay can also be run at basal or maximal insulin concentration. Representative dose-response curves for insulin and IGF-1 are shown in figures 71A-71Z; 71A2-71Z2; 71A3-B3. Qualitative references are shown in Table 14.

TABLE 14

Comp.	Resp.	#expts	ED ₅₀	Comments
1	2	3	4	5
D101	0	4		
D102	0	2		Precipitates
D103	0	2		
D104	0	2		Precipitates
D105	0	2		
D106	0	2		Precipitates
D107	-2	2		
D108	-1	2		
D110	-1	2		
D110	-2	4		
D111	0	2		
D112	0	5		Precipitates
D113	+2	7	Approx 20 µM	Insoluble, especially after freeze-thaw, resulting in inconsistent results. Some response at basal insulin.
D114	0	2		
D115	0	3		
D116	+2	4	> 20 µM	Slight effect at basal insulin
D117	+2	8	Approx 20 μM	Precipitates. Under assay conditions, soluble at least up to 20µM (no ppt in microscope, low magnification). Some response at basal insulin.
D117K	+2	2	> 20 µM	
D118	+2	5	Approx 20 µM	Biphasic dose response curve (needs repeating)
D119	+1	2		
D120	-1	4		
D121	-1	3		
D122	-1	6		
D123	-1	5		Precipitates
D124	0	5		Precipitates
D125	0	2		
D126	0	2		

Includes series "A" e.g. D101A
 Subjective ranking, on a scale of -2 (antagonist) to +2 (agonist)
 Includes experiments run at basal and sub maximal insulin concentrations
 Estimated, not calculated values.

⁵ "Precipitates" indicates precipitate in diluted stock prior to adding to assay. May be soluble under assay conditions

Results:

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The binding assays showed that most of the peptides completely inhibited insulin binding to HIR with IC₅₀-values ranging from 0.3 to 20 μ M. One peptide (D124) was active at lower concentration but only displaced insulin partially (see Figure 71). One peptide (D112) had high affinity for HIGF-1R, but all the others showed 2-20 fold selectivity for HIR (see Figure 71).

In the effect assay (FFC), several of the peptides had no effect, some were antagonists, and a few were agonists reaching a response comparable to that of full insulin stimulation. The ED_{50} for the best peptides (D113 and D117) was around 20-30 μ M.

Despite a right shifted does response curve relative to insulin, these peptides represent the first non-insulin compounds ever found to elicit a maximal insulin response by binding to the insulin receptor. Such peptides may be useful for development as therapeutics themselves.

The peptides could also be useful as leads for further characterization of molecular requirements for binding to and activation of IR, and/or as tools for identification of the mechanisms involved in the activation.

Analysis of affinity and activity of another group of peptides is shown in Table 15. In addition to presenting data on the single chain or looped peptide, Table 15 also reports data showing high affinity binding of certain dimers.

TABLE 15

Name	Sequence	HIR affinity mol/l	FFC
S105	FHENFYDWFVRQV A KK-NH ₂	3.1*10 ⁻⁷	++
S106	FHENFYDWFVRQASKK-NH ₂	4.2*10 ⁻⁷	++
S107	FHENFYDWFVRAVSKK-NH ₂	10.0*10 ⁻⁷	+
S108	FHENFYDWFVAQVSKK-NH ₂	7.5*10 ⁻⁷	+
S109	FHENFYDWFARQVSKK-NH ₂	2.3*10 ⁻⁷	++
S110	FHEAFYDWFVRQVSKK-NH ₂	2.2*10 ⁻⁷	++
S111	FHANFYDWFVRQVSKK-NH ₂	3.3*10 ⁻⁷	0
S112	FAENFYDWFVRQVSKK-NH ₂	6.1*10 ⁻⁷	+
S113	AHENFYDWFVRQVSKK-NH ₂	5.9*10 ⁻⁷	+
S114	fhenfydwfvrqvskk	8.3*10 ⁻⁶	0
S115	EFHENFYDWFVRQVSEE	6.5*10 ⁻⁷	+
S116	FHENFYGWFVRQVSKK	1.4*10 ⁻⁶	++
S117	HETFYSMIRSLAK	2.7*10 ⁻⁶	0
S118	SDGFYNAIELLS	2.4*10 ⁻⁶	+
S119	SLNFYDALQLLAKK	1.8*10 ⁻⁶	0
S120	HDPFYSMMKSLLK	2.0*10 ⁻⁶	0
S121	NSFYEALRMLSSK	3.1*10 ⁻⁶	0
S122	HPTSKEIYAKLLK	9.3*10 ⁻⁶	0
S123	HPSTNQMLMKLFK	1.6*10 ⁻⁵	0
S124	HPPLSELKLFLIKK	2.3*10 ⁻⁵	0
S125	HAPLSVLVQALLKK		0
S126	HPSLSDMRWILLK		
S127	WSDFYSYFQGLD	1.2*10 ⁻⁶	0
S128	D117-Dap(D117)	1.1*10 ⁻⁶	++
S129	SSNFYQALMLLS	2.9*10 ⁻⁶	0
S131	D117-Dap(CO-CH ₂ -O-NH ₂)	1.2*10 ⁻⁶	+
S137	HENFYGWFVRQVSKK	7.7*10 ⁻⁷	0
S145	D117-Lys(D117)	1.5*10 ⁻⁶	++
S147	D117-b-Ala-Lys(D117)	9.3*10 ⁻⁷	++
S148	D117-b-Ala-Dap(b-Ala-D117)	1.1*10 ⁻⁶	++
S149	D117-Gly-Lys(Gly-D117)	2.0*10 ⁻⁶	++
S150	D117-b-Ala-Lys(b-Ala-D117)	6.2*10 ⁻⁷	++
S152	D117-Dab(D117)	5.2*10 ⁻⁶	+
S153	D117-Orn(D117)	3.9*10 ⁻⁶	+
S154	D117-Dap(b-Ala-D117)	3.6*10 ⁻⁶	+
S155	D117-b-Ala-Orn(b-Ala-D117)	2.5*10 ⁻⁶	++
S156	1-(Thia-b-Ala-D117) ₂		
S157	FHENFYDWFVRQVS		
S158	FHENFYDWFVRQVSK	8.1*10 ⁻⁷	+
S159	FHENFYDWFVQVSK		

S160	FHENFYDWFVVSK		
S161	FHENFYDWFVSK		
S162	FHENFYDWFVK		
S165	FYDWF-NH ₂	>2*10 ⁻⁵	0
		>2*10 ⁻⁵	0
S166	FYDWFKK-NH ₂	>2*10	(-)
S167	AFYDWFAKK-NH ₂	3.8*10 ⁻⁶	0
S168	AAAAFYDWFAAAAAKK-NH ₂	5.8*10 ⁻⁷	++
S169	(D117) ₂ - <u>12</u>	7.0*10	
S170	(Cys-Gly-D117) ₂		+++
S171	Cys-Gly-D117	2.9*10-6	+++
S172	(D117) ₂ - <u>14</u>	4.8*10 ⁻⁶	+++
S173	LDALDRLMRYFEERPSL-NH ₂	1.2*10 ⁻⁶	0
S174	PLAELWAYFEHSEQGRSSAH-NH ₂	1.6*10 ⁻⁵	0
S175	GRVDWLQRNANFYDWFVAELG-NH ₂	2.3*10 ⁻⁷	+++
S176	NGVERAGTGDNFYDWFVAQLH-NH ₂	4.7*10 ⁻⁷	+
S177	EHWNTVDPFYFTLFEWLRESG-NH ₂	2.7*10 ⁻⁶	0
S178	EHWNTVDPFYQYFSELLRESG-NH ₂	1.3*10 ⁻⁷	++
S179	QSDSGTVHDRFYGWFRDTWAS-NH ₂	5.4*10 ⁻⁷	+
S180	AFYDWFAK-NH ₂	>2*10 ⁻⁵	0
S181	AFYDWFA-NH ₂	>2*10 ⁻⁵	0
S182	AFYDWF-NH ₂	>2*10 ⁻⁵	0
S183	FYDWDA-NH ₂	>2*10 ⁻⁵	0
S184	Ac-FYDWF-NH ₂	>2*10 ⁻⁵	0
S203	Lig-FHENFYDWFVRQVSKK		
S204	Lig-GGGFHENFYDWFVRQVSKK		
S205	FHENFYDWFVRQVSKKGGG-Lig		
S206	Lig-CAWPTYWNCG		
S207	ACAWPTYWNCG-Lig		
S208	ACAWPTYWNCGGGG-Lig		
S209	Lig-SDGFYNAIELLS		
S210	SDGFYNAIELLS-Lig		
S211	SDGFYNAIELLSGGG-Lig		
S212	KHLCVLEELFWGASLFGYCSGKK-Lig		
S213	AFYDWFAKK-Lig		
S214	AFYEWFAKK-NH ₂	>2*10 ⁻⁵	0
S215	AFYGWFAKK-NH ₂	>2*10 ⁻⁵	0
S216	AFYKWFAKK-NH ₂	>2*10 ⁻⁵	0
S217	(SDGFYNAIELLS-Lig) ₂ -14	3.9*10 ⁻⁸	++
S218	(AFYDWFAKK-Lig) ₂ -14	1,1*10 ⁻⁵	0
S219	FHENAYDWFVRQVSKK	>2*10 ⁻⁵	0
S220	FHENFADWFVRQVSKK	>2*10 ⁻⁵	0
S221	FHENFYAWFVRQVSKK	1.1*10 ⁻⁶	(+)
S222	FHENFYDAFVRQVSKK	>2*10 ⁻⁵	0

S223	FHENFTDWAVRQVSKK	>2*10 ⁻⁵	0
S224	FQSLLEELVWGAPLFRYGTG	>2*10 ⁻⁵	0
S225	PLCVLEELFWGASLFGQCSG		
S226	QLEEEWAGVQCEVYGRECPS	1.6*10 ⁻⁶	
S227	Cys-(Gly) ₂ -D117	5.1*10 ⁻⁷	++
S228	(Cys-(Gly) ₂ -D117) ₂	3.6*10 ⁻⁷	++
S229	(S210)- <u>14</u> -(S212)	4.4*10 ⁻⁹	0
S230	(S131)- <u>14</u> -(S212)		
S231	(S205) ₂ -14	2.7*10 ⁻⁷	+
S232	(S204) ₂ - <u>14</u>	3.8*10 ⁻⁷	+++
S233	(S131)- <u>14</u> -(S210)	2.6*10 ⁻⁷	+
S234	RVDWLQRNANFYDWFVAELG	1.3*10 ⁻⁷	++
S235	VDWLQRNANFYDWFVAELG	5.3*10 ⁻⁸	++
S236	DWLQRNANFYDWFVAELG	1 0*10 ⁻⁷	++
S237	WLQRNANFYDWFVAELG	8.5*10 ⁻⁷	0
S238	LQRNANFYDWFVAELG	8.5*10 ⁻⁷	0
S239	QRNANFYDWFVAELG	1.3*10 ⁻⁶	0
S240	RNANFYDWFVAELG	1.4*10 ⁻⁶	
S241	NANFYDWFVAELG	1 6*10 ⁻⁶	
S242	ANFYDWFVAELG	2.0*10-6	
S243	NFYDWFVAELG	2.0*10 ⁻⁶	
S244	GRVDWLQRNANFYDWFVAELG-Lig	2.2*10 ⁻⁷	++
S245	Lig-GRVDWLQRNANFYDWFVAELG	2.2*10 ⁻⁷	+
S246	(S208)-14-(S131)	5.0*10 ⁻⁶	
S247	(S208)-14-(S209)		
S248	GRVDWLQRNANFYDWFVAEL	6.3*10 ⁻⁸	++
S249	GRVDWLQRNANFYDWFVAE	7.4*10 ⁻⁷	0
S250	GRVDWLQRNANFYDWFVA	8.9*10 ⁻⁶	0
S251	GRVDWLQRNANFYDWFV	5.6*10 ⁻⁶	
S252	14-(SDGFYNAIELLS-Lig) ₂	4.4*10 ⁻⁷	0
S253	(GRVDWLQRNANFYDWFVAELG)-14	2.2*10 ⁻⁸	++
S254	14-(GRVDWLQRNANFYDWFVAE LG)		
S255	(SDGFYNAIELLSGGG) ₂ -14	1.6*10 ⁻⁶	0
S256	H-Acy-CLEE-w-GASL-Tic-QCSG-NH ₂	9.0*10 ⁻⁶	(-)
S257	RWPNFYGYFESLLTHFS-NH ₂	1.4*10 ⁻⁵	0
S258	HYNAFYEYFQVLLAETW-NH ₂		
S259	EGWDFYSYFSGLLASVT-NH ₂	7.7*10 ⁻⁶	0
S260	LDRQFYRYFQDLLVGFM-NH ₂	2.3*10 ⁻⁶	0
S261	WGRSFYRYFETLLAQGI-NH ₂	>2*10 ⁻⁵	0
S262	PLCFLQELFGGASLGGYCSG-NH ₂	1.9*10 ⁻⁵	0
S263	WLEQERAWIWCEIQGSGCRA-NH ₂	>2*10 ⁻⁵	0
S264	IQGWEPFYGWFDDVVAQMFEE-NH ₂	1.9*10 ⁻⁷	0
S265	TGHRLGLDEQFYWWFRDALSG-NH ₂	1.1*10 ⁻⁷	0

S266	H- Abu -CLEE-w-GASL- Tic -QCSG-NH ₂	>2*10 ⁻⁵	0
S267	14-(Dap-CAWPTYWNCG) ₂		
S268	RDHypFYDWFDDi-NH ₂	4.5*10 ⁻⁷	0
S273	S131- <u>14</u> -S209	1.5*10 ⁻⁶	+
S274	S294- <u>14</u> -S210		
S275	S295- <u>14</u> -S210		
S276	S294- <u>14</u> -204		
S277	S295- <u>14</u> -S204		
S278	GFREGQRWYWFVAQVT-NH ₂	>2*10 ⁻⁵	0
S279	VASGHVLHGQFYRWFVDQFALEE-NH ₂		
S280	VGDFCVSHDCFYGWFLRESMQ-NH ₂		
S281	DLRVLCELFGGAYVLGYCSE-NH ₂	1.1*10 ⁻⁵	0
S282	HLSVGEELSWWVALLGQWAR-NH ₂	>2*10 ⁻⁵	0
S283	APVSTEELRWGALLFGQWAG-NH ₂	>2*10 ⁻⁵	0
S284	ALEEEWAWVQVRSIRSGLPL-NH ₂	>2*10 ⁻⁵	0
S285	WLEHEWAQIQCELYGRGCTY-NH ₂	8.3*10 ⁻⁷	
S286	AAVHEQFYDWFADQYEE-NH ₂		
S287	QAPSNFYDWFVREWDEE-NH ₂	5.9*10 ⁻⁶	0
S288	QSFYDYIEELLGGEWKK-NH ₂	4.3*10 ⁻⁶	0
S289	DPFYQGLWEWLRESGEE-NH ₂	>2*10 ⁻⁵	0
S290	(S204) ₂ - <u>7</u>	9.0*10 ⁻⁷	++
S291	(S204) ₂ -9	1.2*10 ⁻⁶	++++
S292	(S204) ₂ -12	7.5*10 ⁻⁷	++
S293	(S204) ₂ - <u>13</u>	1.2*10 ⁻⁷	++
S294	DWLQRNANFYDWFVAEL-Lig	1.3*10 ⁻⁷	++
S295	Lig-DWLQRNANFYDWFVAEL	4.8*10 ⁻⁷	+
S296	(S209) ₂ -9		
S297	(S210) ₂ -9		
S298	LigKHLCVLEELFWGASLFGYCSGKKKK		
S299	KHLCVLEELFWGASLFGYCSGKKKK-Lig		
S300	(S294) ₂ - <u>14</u>	5.0*10 ⁻⁸	+++
S301	(S295) ₂ - <u>14</u>	6.4*10 ⁻⁷	+
S302	S-D-G-F-Y-N-A-Acy-E-L-L-S		
S303	S-G-P-F-Y-E-E-Acy-E-L-L-W-Aib		
S304	G-G-S-F-Y-D-D-Acy-E-Aib-L-W-Aib		
S305	N-Aib-P-F-Y-D-E-Acy-D-E-Cha-W-Aib		
S306	GRVDWLQRNANFYDWFVAEAcyG-NH ₂		

 $\underline{7}$, $\underline{9}$, $\underline{12}$, $\underline{13}$, and $\underline{14}$ represent specific chemical linkers (see Table 18) FFC: 0 is no effect, + is agonist, - is antagonist

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Example 27: Formula 8 synthetic Peptides with Their Affinities for the Human Insulin Receptor (HIR)

A commercial phage display peptide library (New England Biolabs Ph.D.-C7C Disulfide Constrained Peptide Library) was screened for members which bind to IR.

A. Identification of IR Binding Phage

Binding of phage with displayed peptides was detected by ELISA assay. Plates were coated with anti-FC antibody for 2 h at RT or overnight at 4°C. Nonspecific sites were blocked with skim milk (2%) for 1 h at RT. 'sIR-Fc, a modified form of IR in which the cytoplasmic region is substituted with an IgG-Fc fragment (Bass *et al.*, 1990), was then added to the wells for 2 h at RT. Phage were then added to wells and incubated with or without competing peptides for 2 h at RT. Binding was detected with an anti-phage HRP antibody which was added to the wells and incubated for 2.5 h. at RT. OPD (o-phenylenediame) color reaction was detected between 5 and 10 min.

B. Characterization of Phage Displayed Peptides

Fifteen different phage were isolated from a linear 12-mer peptide library (New England Biolabs) panned against a dimer of the LI portion of IR (IR Δ703) (Kristensen *et al.*, 1998) Table 16. The displayed sequences were divided into three groups based on their consensus sequences which correspond to Formula motifs 1, 2 and 7. As can be seen in Table 16, the peptides of motif 7 bind strongly to sIR but not sIGF-1R-FC.

The ability of certain peptides identified in the phage library to compete with other peptides is shown in Table 17 below.

J101 (see Figure 8), the peptide expressed by phage CP42, and containing the Formula 8 motif was found to displace insulin from IR with an IC $_{50}$ of about 5 μm and to be an antagonist in the receptor autophosphorylation and fat cell assays. J101 also does not bind the IR

 Δ 703 construct and is not displaced from IR by insulin. Accordingly, J101, may bind IR outside of the insulin binding site. J101, which contains two cysteine residues is likely to have a cyclic structure.

Phage displaying IR binding peptides were also identified by binding phage to plates coated with sIR-Fc as discussed above and washing away non-binding phages. Binding phage were eluted with glycine-HCl, pH 2.2 for 10 min.

The sequences of the displayed peptides which bind IR are shown in Figure 8.

A few of the peptides (e.g. J101 and J115) (Figure 8) were tested in the fat cell assay and all were full antagonists.

TABLE 16

							-
					Relative binding to	inding to	Formula
IM no.	Isolate	Displayed peptide sequence	No. Found		sIR	sIGF- 1R-Fc	Motif
INAAE	A 10.3 #6.	APTEYAWFNOOT-GGGS.	_	[~J229] IC50~2.4 um	+ + +	+	-
IM447	Λ-12-3 #0. Λ-12-3 #45·	SFYEAIHOLLGV-666S.	23	[~J227]	‡ ‡	‡ ‡ +	7
IM460	Λ-12-3 #76:	NSFYEALRMLSS-GGGS.	2	IC ₅₀ -6,4 µm	‡	(+)	2
IM453	Λ-12-3 #112:	SLNFYDALQLLA-GGGS.	-		++++	‡	5
IM466	∆-12-3* #146	SSNFYQALMLLS-GGGS.			+ + +	‡ :	2 0
IM448	A-12-3 #40	SDGFYNAIELLS-GGGS.	က		+	ŧ)	7
1M446	Λ-12-3 #24	HETFYSMIRSLA-GGGS.	09		++++	+++	2
IM455	Λ-12-3* #10	HDPFYSMMKSLL-GGGS.	-		‡ ‡	‡	2
IM465	Λ-12-3 #193	WSDFYSYFQGLD-GGGS.	-		† + +	(+)	7
>50% Consensus.	.silsua	FY Al L					
							ı
IM452	Λ-12-3 #23:	HPPLEHLKAFLL-GGGS.	4	[~J228]	+ + + +	ſ	~ 1
IM451	∆-12-3 #34:	HPPLSELKLFLI-GGGS.	33	IC ₅₀ ~24 μm S124	+ + + +	ŀ	~ 1
IM459	A-12-3* #60:	HPSLSDMRWILL-GGGS.	2		+ + +	1	<u>~ 1</u>
IM458	∆-12-3* #30:	HAPLSVLQALL-GGGS,	2		‡! ! !	-	/
IM449	∆-12-3 #43:	HPTSKEIYAKLL GGGS.	14		+	I	
IM450	Λ-12-3 #28:	HPSTNQMLMKLF-GGGS.	40		‡	I	7
				S122			
≥50% Consensus:	ensus:	HPPLSL_LL		2123			

TABLE 17

Phage	Sequence Formula Motif	D103	D118 2	D119 2	D120	10 10	0122 10	D123 6	D124 4	Insulin
18/332/~ 1404)	Cvolic	% 85	% 100	% 100	% 100	% 100	% 100	% 100	+71	% 98
1010 JUN 1010	ADTEVAMENOOT) 	++ 2	c ‡	0 ++	0 ++	% 100	% 100	+ 68	++ 11
101440(-0229)	OEVENIHO! CV	†	1 =	0 ++	0 +	0 ‡	% 85	+ 58	+ 46	++3
11V(447(~3227)	HDDI FHI KAFI I	1	; ;	++ 10	0 ++	0++	% 95	pu	% 84	+ 26
IM242(~3220)		+37	++ 17	+46	+30	99+	+57	+55	++ 19	0 ++

%:>80% signal (not displaced) +: 20-70% signal ++: <20% signal (fully displaced)

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EXAMPLE 28: PREPARATION OF THE DIMERS

A. Materials

Generally, suitably protected N-Fmoc (fluorenylmethoxycarboxyl)amino acids were purchased from Novabiochem (Switzerland), 1-hydroxy-7azabenzotriazole (HOAt) from Perspective Biosystems and N,N'diisopropylcarbodiimide (DIC) from Fluka. The molecular weights of the peptides were determined using matrix-assisted laser desorption time offlight mass spectroscopy (MALDI-MS), recorded on a Voyager-DE (Perseptive Biosystems). A matrix of sinapinic acid was used. Analytical and semi-preparative high-pressure liquid chromatography (HPLC) were performed using a Waters RCM 8 x 10 module and with a C-18 column (19 x 300 mm) and a C-18 column (25 x 300 mm), respectively, at 40° C. The solvent system for both analytical and semi-preparative HPLC was buffer A; 0.1% TFA in water and buffer B; 0.07% TFA in 100% and UV detection was at 215 nm. The gradient for analytical HPLC (1.5 ml/min); a linear gradient of 5-90% buffer B over 25 min and semi-preparative HPLC (4 ml/min); an isocratic gradient of 20% buffer B over 5 min, followed by a linear gradient of 20-60% buffer B over 40 min.

B. Solid-Phase Peptide Synthesis and Analysis of the D117 Monomer(FHENFYDWFVRQVSKK-Dap(CO-CH2-O-NH2)

The peptide monomer available for ligation was synthesized manually in plastic syringes using a preloaded Rink amide linker (RAM)-TentaGel (0.26 mmol/g). Fully protected N-Fmoc amino acids (3 equiv.) were used and the temporary Fmoc protecting group was removed after each cycle by 30% piperidine in *N*-methylpyrrolidone (NMP). The natural amino acids were coupled as their free acids in NMP using DIC (3 mol equiv.) and HOAt (3 mol equiv.) as coupling additive.

First, Fmoc-Dap(Alloc) was coupled as described above. The alloc group was then removed by Pd(0) (3 mol equiv.) in CHCl₃/AcOH/N-

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methylmorpholine (37:2:1, v/v/v) under helium. After 2 h. at RT, the resin was washed with 5% in NMP containing 2% diethyldithiocarbamide, Na salt. Finally, the resin was washed with NMP containing HOBt (hydroxybenzotriazole). The protected oxyamino acetic acid (3 mol equiv.) was then coupled on the side-chain of Dap (diaminopropionic acid). The completion of all the acylation reactions was monitored visually by the use of bromophenol blue. Between the Fmoc-deprotection and the acylation reaction, the resin was washed with NMP (x 6).

After synthesis, the peptide was washed with DCM (dichloromethane) (x 3). The peptides were cleaved simultaneously from the resin and the side-chain protecting groups were removed by treatment with 95% aqueous TFA containing triisopropylsilan (TIS) (4 molar equiv.) for 1.5 h. The resin was rinsed with 95% aqueous acetic acid (x 4). Both TFA and acetic acid were evaporated and the peptide was finally precipitated in diethyl ether and lyophilized overnight. The peptide was both analyzed by analytical HPLC and MALDI-MS. Analysis by MALDI-MS; m/z 2287.5 (M + H)+ (requires m/z, 2288.3) confirmed the expected product.

To the peptide monomer, FHENFYDWFVRQVSKK-Dap(CO-CH2-O-NH2) (9.1 mg, 3.9 mol) was added the dialdehyde linker (0.81 mol) dissolved in 80% DMSO (aqueous) (28 l). The pH was then adjusted to 5 with solid sodium acetate. The solution was left overnight at 37°C and progress of the reaction was monitored by RP-HPLC. The formed dimer (see Table 18) was purified by semi-preparative HPLC. Analysis by MALDI-MS confirmed the expected product (see Table 18). The molecular weights and inter peptide distance of various linkers is shown below.

TABLE 18

Structure	Number	MW	MW (- 2H ₂ O)
0 0	1	100.1	64.1
0 0	<u>2</u>	58.04	22.04
	3	149.15	113.15
	4	150.14	114.14
	5	134.13	98.13
	<u>6</u>	134.13	98.13
	7	134.13	98.13
	8	234.25	198.25
	9	302.3	266.3
0	10	72.06	36.06
	11	86.09	50.09
	12	114.14	78.14
	13	128.08	92.08
·///	14	142.19	106.19

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Dimers were prepared by ligation chemistry (oxime bond in the ligation site) (attached through C-terminal domain).

C. Binding of Dimers to Different IR Constructs Indicates Peptides Bind to Two Independent Sites

Table 19 summarizes the results of binding of phages of D117 (Formula 1 Motif), D123 (Formula 6 motif), D124 (Formula 4 motif), and CP42 (phage expressing peptide J101, Formula 8 motif) monomer to constructs of IR consisting of the L1-cys-L2 region, L1-cys-L2-FnIIIα region and L2-FnIIIα region.

10 <u>TABLE 19</u>

IR Construct	Peptides Bound	Motif
L1-cys-L2	D117	A6 Only
L1-cys-L2-FnIIIα	D117, CP42, D123, 124	A6, D8, F8
L2-FnIIΙα	CP42, D123, D124	D8, F8

The data above is consistent with a conclusion that the A6 (Formula 1 motif) and F8 (Formula 4 Motif) motifs are physically distinct and on separate parts of IR. Competition data, supra, further indicates that the binding site for the B6 (Formula 2 motif) is on the same subunit as that for the A6 motif.

As shown below, BIAcore competition studies are consistent with the separation of Sites 1 (A6, B6) and 2 (D8, F8, J101).

20 D. Competition of Site 1 and Site 2 Phage Displayed Peptides with Recombinant Cleaved Di-Peptides

Insulin receptor was coated on a 96-well plate with 50 μ l of a 2 ng/ μ l solution of IR and incubated overnight at 4°C. The wells were then blocked with MPBS for 1 h.

Dimers were prepared by expressing them as MBP fusion products.

See, Table 1, supra. The sequences of the MBP- cleaved dimers are shown below (core peptide sequences are underlined):

Cleaved Dimer Sequences

5 **#426 (D8)**AQPAMA<u>WLDQEWAWVQCEVYGRGCPS</u>AAAGAPVPYPDPLEPRAA.

#429(D8-6-D8)

AQPÀMAWLDQEWAWVQCEVYGRGCPSGGSGSWLDQEWAWVQCEVY 10 GRGCPSAAAGAPVPYPDPLEPRAA.

#459 (short flag RB6)
ISEFGSADYKDLDALDRLMRYFEERPSLAAAGAPVPYPDPLEPRAA.

15 **#430 (H2C-4-RB6)**DYKDDDD<u>KFHENFYDWFVRQVSGSGSLDALDRLMRYFEERPSL</u>AAAGAP
VPYPDPLEPRAA.

#464 (H2C)

20 DYKDDDDFHENFYDWFVRQVSAAAGAPVPYPDPLEPRAA.

#446 (F8)
DYKDDDD<u>HLCVLEELFWGASLFGYCSG</u>AAAGAPVPYPDPLEPRAA.

25 **#431 (H2C-6-F8)**DYKDDDD<u>KFHENFYDWFVRQVSGGSGSHLCVLEELFWGASLFGYCSG</u>
AAAGAPVPYPDPLEPRAA.

#433 (H2C-9-F8)

30 DYKDDDD<u>KFHENFYDWFVRQVSGGSGGSGSHLCVLEELFWGASLFGY</u> CSGAAGAPVPYPDPLEPRAA.

#432 (H2C-12-F8)

DYKDDDD<u>KFHENFYDWFVRQVSGGSGGSGGSGSHLCVLEELFWGASL</u> 35 <u>FGYCSG</u>AAAGAPVPYPDPLEPRAA.

#452 (G3)
AQPAMARGGGTFYEWFESALRKHGAGAAAGAPVPYPDPLEPRAA.

40 #427 (G3-6-G3)
AQPAMARGGGTFYEWFESTLRKHGAGGGSGGSRGGGTFYEWFESALRK
HGAGAAAGAPVPYPDPLEPRAA.
(* A TO T CHANGE)

#428 (G3-12-G3)

AQPAMARGGGTFYEWFESALRKHGAGGGSGGSGGSGGSRGGGTFYEW FESALRKHGAGAAAGAPVPYPDPLEPRAALTN.

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#434 (G3-12-G3)

ISEFIÈVRAQPAMARGGGTFYEWFESALRKHGAGGGSGGSGGSGGSRGGTFYEWFESALRKHGAGAAAGAPVPYPDPLEPRAA.

10 #437 (H2C)

AQPAMAFHENFYDWFVRQVSAAAGAPVPYPDPLEPRAA.

#463 (H2C-3-H2C)

15 AQPAMA<u>FHENFÝDWFVRQVSGGSFHENFYDWFVRQVS</u>AAAGAPVPYPD PLEPRAA.

#435 (H2C-3-H2C-3-H2C)

AQPAMA<u>FHENFYDWFVRQVSGGSFHENFYDWFVRQVSGGSFHENFYD</u>
20 WFVRQVSAAAGAPVPYPDPLEPRAA.

#439 (H2C-6-H2C)

AQPAMA<u>FHENFYDWFVRQVSGGSGGSFHENFYDWFVRQVS</u>AAAGAPVP YPDPLEPRAA.

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#436 (H2C-9-H2C)

AQPAMAFHENFYDWFVRQVSGGSGGSGGSFHENFYDWFVRQVSAAAG APVPYPDPLEPRAA.

30 **#449 (H2C-12-H2C)**

AQPAMA<u>FHENFYDWFVRQVSGGSGGSGGSGGSFHENNFYDWFVRQVS</u> AAAGAPVPYPDPLEPRAA.

MBP*

35 ISEFGSSRVDLQASLALAVVLQRRDWENPGVTQLNRLAAHPPFASWRNSEEA RTDRPSQQLRSLNGEWQLGCFGG

The MBP- cleaved fusion protein mixtures were appropriately diluted, added to the wells, and incubated at RT for 30 min. An equal volume of F8 or H2C phage displayed peptide was then added to each well and incubated for 1 h. The control wells (100% phage binding) contained only phage and an equal volume of buffer. The control cleaved fusion protein mixture contains a peptide derived from the lacZ gene. The plate was washed 3

times in PBST and then incubated with HRP/anti-M13 conjugate for 45 min. The plate was washed again and then the ABTS substrate added. The values indicate readings taken at OD_{405} . Figure 72A shows competition between cleaved monomers and dimers and F7 phage for binding to Site 2 of IR. Figure 72B shows competition for binding to Site 1 between H2C and the cleaved and uncleaved monomers and dimers. IC_{50} values are shown in Table 20.

TABLE 20

Dimers Site 1/Site 2	2 IC ₅₀ Values			
Phage Signal	H	2C	F	8
Cleavage	-	+	-	+
Dimers				
LF-H2C(6)F8	0.2	0.19	0.3	5
LF-H2C(9)F8	0.4	0.11	3	15
LF-H2C(12)F8	0.3	0.19	>16	16
LF-F8 mono	-	-	>20	12
LF-H2C mono	0.145	0.11	>1	>1
H2C mono	0.3	G.2	>0.5	>0.5
MBP-lacZ control	_	-	-	-

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E. Stimulation of autophosphorylation of IR by MBP-Fusion Peptides

Fusion peptides were prepared as described above, and then assayed for IR activation (see Example 20). The results of these experiments shown in Figure 74 indicate that the H2C monomer and H2C-H2C homodimers stimulate autophosphorylation of IR *in vivo*.

H2C dimers (Site 1-Site 1) with a 6 amino acid linker (H2C-6-H2C) were most active in the autophosphorylation assay. Other active dimers are also shown in Figure 74, particularly H2C-9-H2C, H2C-12-H2C, H2C-3-H2C, and F8.

^{- =} uncleaved

^{+ =} cleaved

Example 29: IGF-1R Peptide Assays

A. IC₅₀ Determinations

Peptides that meet the proper criteria of affinity, selectivity, and activity may be used to develop site-directed assays to identify active molecules which bind to sites on IGF-1R. Assays have been developed using Time-Resolved Fluorescence Resonance Energy Transfer (FRET). These assays are not radioactive, homogeneous (no wash steps), and can be rapidly carried out in 96- or 384-well microtiter plate format facilitating their use in high-throughput screening assays for small organic molecules.

This assay can be used to assess the affinity of peptides for IGF-1R or can be used to find small organic molecule leads in a high-throughput capacity. The determination of the IC_{50} for several peptides is described below.

1. Assay Components

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IGF-1R was purchased from R&D System, Cat. # 391-GR/CF. The IGF-1R was labeled with Europium (Eu) by EG&G Wallac. Ten milligrams of IGF-1R was sent to Wallac and the IGF-1R was labeled with Wallac's W-1024 Eu-chelate.

The Streptavidin-Allophycocynanin (SA-APC) was obtained from Prozyme Cat. # PJ25S. The biotinylated 20E2
[DYKDFYDAIDQLVRGSARAGGTRDKK(ε-biotin)] ("b-20E2") was synthesized by Novo Nordisk or by PeptidoGenic Research & Co., Inc. The IGF-1 was commercially available from PeproTech Cat. # 100-11.

2. Assay Method

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a. Preparation of the Assay Mix. A 2X concentration of Assay Mix consisting of 4 nM Eu-labeled IGF-1R, 30 nM b-20E2, 4 nM SA-APC, and 0.1% BSA was prepared. This mixture was allowed to pre-incubate at RT in the dark for 1-2 h before competitor was added.

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- b. Dilutions of the Competitors were carried out on a 96-well microtiter plate (Costar Cat. #3912). 100 μ l of Buffer (TBS pH 8.0 + 0.1 % BSA) were dispensed to wells in columns 1 through 11. Competitors and Buffer were added to Column 12 wells so that the total volume is 150 μ l.
- c. To identify small organic compounds which also bind the active sites of IGF-1R, dilutions of the small organic compounds are also performed on a 96-well microtiter plate (Costar Cat. #3912). Compounds are dissolved in 100% DMSO. Therefore, 100 μ l of Buffer (TBS pH 8.0 + 0.1 % BSA) with 4% DMSO are dispensed to wells in columns 1 through 10. Column 11 contains 100 μ l of Buffer with 2.7% DMSO. Compounds (6 μ l) are added into 144 μ l Buffer (No DMSO) to Column 12 wells.
- d. Dilutions were performed across columns on the plate. Once competitors were dispensed into Column 12 and mixed, 50 µl of the solution Column 12 were transferred to wells in 11 and mixed. 50 µl of the Column 11 mixture was transferred to Column 10 wells. This was repeated until 50 µl of Column 3 mixture was transferred to Column 2 wells. Once accomplished to Column 2, 50 µl from Column 2 were removed and discarded. Column 1 wells were reserved for No Competitor Wells. 100 µl volume was therefore maintained across all columns.
- e. 50 μ l of the Assay Mix was dispensed into wells on a new 96-well microtiter plate. 50 μ l from the Dilutions Plate were then added to this plate.
- f. 30 μ l from Assay Mix Plate were transferred from the 96-well in duplicate on a 384-well microtiter plate (Nunc Cat. # 264512). This covered plate was allowed to incubate at RT overnight.
- g. Binding was measured using Wallac's Victor II fluorometer by excitation at 340 nm and measuring emission at 665 and 615 nm.
- h. The working concentrations of this assay were 2 nM Eulabeled IGF-1R, 15 nM b-20E2, 2 nM SA-APC, and 0.1% BSA. Peptides were normally diluted starting from 100 μ M, where IGF-1 begins at 30 μ M.

Compounds begin at 200 μM in a working concentration of 2% DMSO. Controls also contained 2% DMSO.

3. Results

The IC₅₀ and holoenzyme phosphorylation activity (see Example 20) values for certain peptides are shown below.

Peptide Data

<u>Name</u>	<u>Sequence</u>	IGF-1R IC ₅₀	Holo. Phos.
IGF-1	Natural Ligand	~1-10 nM	
C1	A6S-4-C1-IGFR or D112	~10 nM	
RP9	H2C Design	33 nM	++
20E2	R20a-3-20E2-IR or D118	~100 nM	
G8	20E2B-3-C6-IGFR	139 nM	-
RP2	H2CB-3-B9-IR	163 nM	+
E8	R20b-4-E8-IR or D120	175 nM	
G33	H2CA-4-G9-IGFR	178 nM	+++
RP6	20C-4-G3-IGFR	184 nM	++++
RP14	H2CA-4-H8-IGFR	225 nM	
S178	B6C-3-C10-IR	240 nM	
RP10	20E2 Design	315 nM	+
S176	A6S-4-G1-IR	418 nM	
H2C	A6S-4-H2-IGFR or D117	~600 nM	+
B6	R40-3-B6-IGFR	631 nM	
RP13	H2CA-4-H6-IGFR	818 nM	
G8	20E2B-3-C6-IGFR	1330 nM	-
S174	R20-4-F9-IGFR	1460 nM	
RP8	20E2 Design	1800 nM	+
S177	B6C-3-C7-IR	2040 nM	
S175	A6S-3-E12-IR	2050 nM	++
RP1	H2CB-4-G11-IR	2790 nM	+
bS175	A6S-3-E12-IR	3230 nM	
NG C2	20E2-3-C2-IGFR	4020 nM	
S179	H2CBa-3-B12-IR	5350 nM	

S173	rB6-4-A12-IR	5620 nM	
RP5	20E2B-3-B3-IR	7450 nM	-
G9	20E2B-1-A6-IGFR	7550 nM	-
RP4	20E2A-4-F9-IR	8110 nM	+
D8 (B12)	D820-4-B12-IR	11300 nM	
RP24	R20b-4-A4-IR	17800 nM	
RP11	A6S Design	18800 nM	+
D8	R20b-4-D8-IR	21650 nM	
A6	R40-3-A6-IGFR	46600 nM	
RP17	R20b-4-A6-IR	50000 nM	
S167	Short A6	~100 µM	
RP3	20E2A-3-B11-IR	~100 µM	-
KC F9	D820-4-F9-IR	~100 µM	
JB3	CONTROL	~100 µM	
KC G1	D820-4-F10-IGFR	~100 µM	
C3-MDM2	CONTROL	>100 µM	
RP21	40F-4-C1-IGFR	>100 µM	
RP22	40F-4-D10-IGFR	>100 µM	
RP23	40F-4-C1-IR	>100 µM	
KC G2	D820-4-F10-IGFR	>100 µM	
KC G7	F815-4-G7-IGFR	>100 µM	
110 07	1010-4-07-10110		

B. IGF-1R Peptide Assay Competition Dissociation

A competition dissociation experiment was performed to determine if any peptides altered the dissociation rate of the 20E2 (B6 motif) peptide in the IGF-1R Peptide Assay. An alteration of the dissociation rate suggests the peptide used in the competition binds to a second site on IGF-1R thus enhancing or slowing the 20E2 dissociation rate through an allosteric interaction.

1. Materials

IGF-1R was purchased from R&D System, Cat. # 391-GR/CF. The

10 IGF-1R was labeled with Europium (Eu) by EG&G Wallac. Ten milligrams of

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IGF-1R was sent to Wallac and the IGF-1R was labeled with Wallac's W-1024 Eu-chelate.

The Streptavidin-Allophycocynanin (SA-APC) obtained from Prozyme Cat. # PJ25S. The biotinylated 20E2

5 [DYKDFYDAIDQLVRGSARAGGTRDKK(ε-biotin)] was synthesized by Novo Nordisk or by PeptidoGenic Research & Co., Inc. The IGF-1 was commercially available from PeproTech Cat. # 100-11.

2. Methods

- a. Preparation of the Assay Mix. A 1.25X concentration of Assay
 Mix consisting of 2.5 nM Eu-labeled IGF-1R, 18.75 nM b-20E2, 2.5 nM SA-APC, and 0.1% BSA was prepared. This mixture was allowed to preincubate.
 - b. 20 µl of Competitor and Buffer were added to a 96-well microtiter plate (Costar Cat. #3912).
 - c. Wallac Victor II Fluorometer was readied to read at 665 nm only in multiple repeats (99) of only the wells containing material.
 - d. 80 µl of the 1.25X Assay Mix was added to the 96-well microtiter plate and promptly placed onto the Victor II for readings.
 - e. After the original 99 repeat readings were taken, periodic readings were taken until equilibrium had been established.

NOTE: Different conditions can be used for these experiments. For example, a 1.1X concentration of assay mix can be initially made. Then first add 10 μ I of Competitor and Buffer to the microtiter plate followed by 90 μ I of the Assay Mix.

f. The working concentrations of this assay were 2 nM Eulabeled IGF-1R, 15 nM b-20E2, 2 nM SA-APC, and 0.1% BSA. Peptides were normally competed at 100 μ M, whereas IGF-1 was competed at 30 μ M. Results are shown in Figure 14.

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3. Results

Figure 14 shows the results of one of the experiments. Clearly, IGF-1 and D8 (B12) cause a much slower dissociation rate than the 20E2 (motif 2), H2C (motif 1), C1 (motif 1), and RP6 (motif 2) peptides. This suggests that IGF-1 and D8 (B12) contact IGF-1R in different locations than that of 20E2, H2C, C1, and RP6.

Previous data (EXAMPLE 28) suggests that the motif 6 series binds to a location of IGF-1R that differs from motifs 1 and 2 and that these two sites are not independent of one another. The slowing of the dissociation rate by IGF-1 and D8 (B12) further suggests that there are at least two sites of binding to IGF-1R and that these two sites are not independent of one another.

The following publications, some of which have been cited herein, are cited for general background information and are incorporated by reference in their entirety.

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We claim:

- 1. A method of modulating insulin activity in mammalian cells, said method comprising administering to said cells an amino acid sequence which binds IR and comprises the amino acid sequence $X_1X_2X_3X_4X_5$, wherein X_1 , X_2 , X_4 , and X_5 are aromatic amino acids, and X_3 is any polar amino acid.
- 2. The method according to claim 1 wherein X_1 , X_2 , and X_5 are selected from the group consisting of phenylalanine and tyrosine, X_3 is selected from the group consisting of aspartic acid, glutamic acid, glycine and serine, and X_4 is selected from group consisting of tryptophan, tyrosine and phenylalanine.
- The method according to claim 2 wherein said amino acid sequence is an insulin agonist.
 - 4. The method according to claim 2 wherein said amino acid sequence is an insulin antagonist.
- The method according to claim either one of claims 3 or 4 wherein X₁ and
 X₅ are phenylalanine and X₂ is tyrosine.
 - 6. The method according to claim 5 wherein X_4 is tryptophan.
 - 7. The method according to claim 6 wherein the amino acid sequence is an insulin agonist and X₃ is selected from the group consisting of aspartic acid and glutamic acid.
- 20 8. The method according to claim 7 wherein X₃ is aspartic acid to result in an amino acid sequence comprising FYDWF.

- 9. The method according to claim 7 wherein X₃ is glutamic acid to result in an amino acid sequence comprising FYEWF.
- 10. The method according to claim 1 wherein the amino acid sequence FHEN is bound to the amino terminal of $X_1X_2X_3X_4X_5$ to produce an amino acid sequence comprising FHENX $_1X_2X_3X_4X_5$ and possessing insulin agonist activity.
- 11. The method according to claim 10 wherein the amino acid sequence is FHENFYDWF.
- 12. The method according to claim 1 wherein the amino acid sequence $X_1X_2X_3X_4X_5$ further comprises the amino acid sequence X_{93} X_{94} X_{95} X_{96} X_{97} located at the carboxy terminal end adjacent to X_5 , wherein X_{93} , X_{94} and X_{97} may be any amino acid, X_{95} is selected from the group consisting of glutamine, glutamic acid, alanine and lysine, and X_{96} is a hydrophobic or aliphatic amino acid.
- 13. The method according to claim 12 wherein X₉₃ is selected from the group consisting of alanine, aspartic acid, glutamic acid, arginine, and valine, X₉₅ is glutamine or glutamic acid, and X₉₆ is selected from the group consisting of leucine, isoleucine, valine and tryptophan.
 - 14. The method according to claim 13 wherein X_{96} is leucine or tryptophan.
- 20 15. The method according to claim 14 wherein X_{96} is leucine.
 - 16. The method according to claim 13 wherein X_{95} is glutamine or glutamic acid, and X_{96} is tryptophan.

- 17. The method according to claim 13 wherein X_{95} is glutamic acid and the amino acid sequence is an insulin agonist.
- 18. The method according to claim 13 wherein asparagine is present as the amino acid bound to the amino terminal of X_1 and wherein $X_1X_2X_3X_4X_5X_{93}$ is FYDWFV
- 19. The method according to claim 1 wherein the amino acid sequence is selected from the group of amino acid sequences listed in Figures 1, 2, and 9.
- 20. The method according to claim 1 wherein the sequence is selected from the group consisting of FHENFYDWFVRQVSK,

 DYKDVTFTSAVFHENFYDWFVRQVSKK,

 GRVDWLQRNANFYDWFVAELG and APTFYAWFNQQT.
 - 21. The method according to claim 1 wherein the sequence is selected from the group consisting of
- 15 FHENFYDWFVRQVAKK-NH₂
 FHENFYDWFVRQASKK-NH₂
 FHENFYDWFVRAVSKK-NH₂
 FHENFYDWFVAQVSKK-NH₂
 FHENFYDWFARQVSKK-NH₂
 FHEAFYDWFVRQVSKK-NH₃
- 20 FHEAFYDWFVRQVSKK-NH₂ FHANFYDWFVRQVSKK-NH₂ FAENFYDWFVRQVSKK-NH₂ AHENFYDWFVRQVSKK-NH₂
 - fhenfydwfvrqvskk
- 25 EFHENFYDWFVRQVSEE FHENFYGWFVRQVSKK HETFYSMIRSLAK SDGFYNAIELLS

SLNFYDALQLLAKK

30 HDPFYSMMKSLLK

	NSFYEALRMLSSK
	HPTSKEIYAKLLK
	HPSTNQMLMKLFK
	HPPLSELKLFLIKK
5	HAPLSVLVQALLKK
	HPSLSDMRWILLK
	WSDFYSYFQGLD
	D117-Dap(D117)
	SSNFYQALMLLS
10	D117-Dap(CO-CH ₂ -O-NH ₂)
	HENFYGWFVRQVSKK
	D117-Lys(D117)
	D117-b-Ala-Lys(D117)
	D117-b-Ala-Dap(b-Ala-D117)
15	D117-Gly-Lys(Gly-D117)
	D117-b-Ala-Lys(b-Ala-D117)
	D117-Dab(D117)
	D117-Orn(D117)
	D117-Dap(b-Ala-D117)
20	D117-b-Ala-Orn(b-Ala-D117)
	$\underline{1}$ -(Thia-b-Ala-D117) ₂
	FHENFYDWFVRQVS
	FHENFYDWFVRQVSK
	FHENFYDWFVQVSK
25	FHENFYDWFVVSK
	FHENFYDWFVSK
	FHENFYDWFVK
	FYDWF-NH ₂
	FYDWFKK-NH ₂
30	$AFYDWFAKK-NH_2$
	AAAAFYDWFAAAAAKK-NH ₂
	$(D117)_2 - \underline{12}$
	$(Cys-Gly-D117)_2$
	Cys-Gly-D117
35	$(D117)_2 - \underline{14}$
	LDALDRLMRYFEERPSL-NH ₂
	PLAELWAYFEHSEQGRSSAH-NH ₂
	GRVDWLQRNANFYDWFVAELG-NH ₂
	NGVERAGTGDNFYDWFVAQLH-NH ₂
40	EHWNTVDPFYFTLFEWLRESG-NH ₂
	EHWNTVDPFYQYFSELLRESG-NH ₂
	QSDSGTVHDRFYGWFRDTWAS-NH ₂
	AFYDWFAK-NH ₂

	AFYDWFA-NH ₂
	AFYDWF-NH ₂
	FYDWDA-NH ₂
	Ac-FYDWF-NH ₂
5	Lig-FHENFYDWFVRQVSKK
	Lig-GGGFHENFYDWFVRQVSKK
	FHENFYDWFVRQVSKKGGG-Lig
	Lig-CAWPTYWNCG
	ACAWPTYWNCG-Lig
10	ACAWPTYWNCGGGG-Lig
	Lig-SDGFYNAIELLS
	SDGFYNAIELLS-Lig
	SDGFYNAIELLSGGG-Lig
	KHLCVLEELFWGASLFGYCSGKK-Lig
15	AFYDWFAKK-Lig
	AFYEWFAKK-NH ₂
	AFYGWFAKK-NH ₂
	AFYKWFAKK-NH ₂
	(SDGFYNAIELLS-Lig) ₂ - <u>14</u>
20	(AFYDWFAKK-Lig) ₂ - <u>14</u>
	FHENAYDWFVRQVSKK
	FHENFADWFVRQVSKK
	FHENFYAWFVRQVSKK
2.5	FHENFYDAFVRQVSKK
25	FHENFTDWAVRQVSKK
	FQSLLEELVWGAPLFRYGTG
	PLCVLEELFWGASLFGQCSG
	QLEEEWAGVQCEVYGRECPS
20	Cys-(Gly) ₂ -D117
30	$(Cys-(Gly)_2-D117)_2$
	(S210)- <u>14</u> -(S212)
	(S131)- <u>14</u> -(S212)
	$($205)_2 - \frac{14}{14}$
25	(\$204) ₂ - <u>14</u>
35	(\$131)- <u>14</u> -(\$210)
	RVDWLQRNANFYDWFVAELG
	VDWLQRNANFYDWFVAELG
	DWLQRNANFYDWFVAELG
40	WLQRNANFYDWFVAELG
→ ∪	LQRNANFYDWFVAELG
	QRNANFYDWFVAELG RNANFYDWFVAELG
	NANFYDWFVAELG NANFYDWFVAELG
	MAINT I DWY V AELU

ANFYDWFVAELG **NFYDWFVAELG** GRVDWLORNANFYDWFVAELG-Lig Lig-GRVDWLQRNANFYDWFVAELG 5 (S208)-14-(S131) (S208)-14-(S209) GRVDWLQRNANFYDWFVAEL GRVDWLQRNANFYDWFVAE GRVDWLQRNANFYDWFVA 10 GRVDWLQRNANFYDWFV 14-(SDGFYNAIELLS-Lig)₂ (GRVDWLQRNANFYDWFVAELG)-14 14-(GRVDWLQRNANFYDWFVAE LG) (SDGFYNAIELLSGGG)₂-14 15 H-Acy-CLEE-w-GASL-Tic-QCSG-NH₂ RWPNFYGYFESLLTHFS-NH₂ HYNAFYEYFQVLLAETW-NH2 EGWDFYSYFSGLLASVT-NH₂ LDRQFYRYFQDLLVGFM-NH₂ 20 WGRSFYRYFETLLAQGI-NH2 PLCFLQELFGGASLGGYCSG-NH₂ WLEQERAWIWCEIQGSGCRA-NH2 IQGWEPFYGWFDDVVAQMFEE-NH2 TGHRLGLDEQFYWWFRDALSG-NH₂ 25 H-Abu-CLEE-w-GASL-Tic-QCSG-NH₂ 14-(Dap-CAWPTYWNCG)2 RDHypFYDWFDDi-NH2 S131-14-S209 S294-14-S210 30 S295-14-S210 S294-14-204 S295-<u>14</u>-S204 GFREGQRWYWFVAQVT-NH2 VASGHVLHGQFYRWFVDQFALEE-NH2 35 VGDFCVSHDCFYGWFLRESMQ-NH₂ DLRVLCELFGGAYVLGYCSE-NH2 HLSVGEELSWWVALLGQWAR-NH₂ APVSTEELRWGALLFGQWAG-NH2 ALEEEWAWVQVRSIRSGLPL-NH2 40 WLEHEWAQIQCELYGRGCTY-NH2 AAVHEOFYDWFADQYEE-NH₂ QAPSNFYDWFVREWDEE-NH₂

QSFYDYIEELLGGEWKK-NH₂

DPFYQGLWEWLRESGEE-NH2 $(S204)_2-7$ $(S204)_2-9$ (S204)₂-12 5 $(S204)_2 - 13$ DWLQRNANFYDWFVAEL-Lig Lig-DWLQRNANFYDWFVAEL $(S209)_2-9$ (S210)₂-9 LigKHLCVLEELFWGASLFGYCSGKKKK 10 KHLCVLEELFWGASLFGYCSGKKKK-Lig (S294)₂-14 $(S295)_2-14$ S-D-G-F-Y-N-A-Acy-E-L-L-S S-G-P-F-Y-E-E-Acy-E-L-L-W-Aib 15 G-G-S-F-Y-D-D-Acy-E-A1b-L-W-Aib N-A1b-P-F-Y-D-E-Acy-D-E-Cha-W-Aib GRVDWLQRNANFYDWFVAEAcyG-NH2 and wherein underlined numbers represent a linker as defined in Table 18.

- 20 22. The method according to claim 2 wherein the amino acid sequence binds to the insulin receptor with an affinity of at least about 10⁻⁵ M.
 - The method according to claim 22 wherein the affinity is at least about 10⁻⁷
 M.
- 24. The method according to claim 23 wherein the affinity is at least about 10⁻⁹

 M.
 - 25. An amino acid sequence comprising X₁X₂X₃X₄X₅ wherein X₁, X₂, X₄, and X₅ are aromatic amino acids, X₃ is any polar amino acid, and wherein said amino acid sequence binds to IGF-1R.
- The amino acid sequence according to claim 25 wherein the IGF-1R binding occurs with an affinity (K_d) of at least about 10⁻⁵ M.

- 27. The amino acid sequence according to claim 25 wherein the binding occurs at an affinity (K_d) of at least about 10^{-7} M.
- 28. The amino acid sequence according to claim 25 wherein X_1 , X_2 , and X_5 are selected from the group consisting of phenylalanine and tyrosine, X_3 is selected from the group consisting of aspartic acid, glutamic acid, glycine and serine, and X_4 is selected from group consisting of tryptohpan, tyrosine and phyenylalanine.
- 29. The amino acid sequence according to claim 28 wherein X_3 is selected from the group consisting of aspartic acid and glutamic acid.
- 10 30. The amino acid sequence according to claim 29 wherein X_1 and X_5 are phyenylalanine and X_2 is tyrosine.
 - 31. The amino acid sequence according to claim 29 wherein X_4 is tryptophan.
 - 32. The amino acid sequence according to claim 31 wherein X_3 is aspartic acid to result in an amino acid sequence comprising FYDWF.
- 15 33. The amino acid sequence according to claim 31 wherein X₃ is glutamic acid to result in an amino acid sequence comprising FYEWF.
 - 34. The amino acid sequence according to claim 28 wherein the amino acid sequence FHEN is bound to the amino terminal of $X_1X_2X_3X_4X_5$ to produce an amino acid sequence comprising FHENX₁X₂X₃X₄X₅.
- 20 35. The amino acid sequence according to claim 34 wherein the amino acid sequence comprises FHENFYDWF.

- 36. The amino acid sequence according to claim 25 wherein the amino acid sequence $X_1X_2X_3X_4X_5$ further comprises the amino acid sequence X_{93} X_{94} X_{95} X_{96} X_{97} located at the carboxy terminal end adjacent to X_5 to form $X_1X_2X_3X_4X_5X_{93}X_{94}X_{95}X_{96}X_{97}$, wherein X_{93} , X_{94} and X_{97} may be any amino acid, X_{95} is selected from the group consisting of glutamine, glutamic acid, alanine and lysine, and X_{96} is a hydrophobic or aliphatic amino acid.
- 37. The amino acid sequence according to claim 36 wherein X₉₃ is selected from the group consisting of alanine, aspartic acid, glutamic acid, arginine, and valine, X₉₅ is glutamine or glutamic acid, and X₉₆ is selected from the group consisting of leucine, isoleucine, valine and tryptophan.
- 38. The amino acid sequence according to claim 37 wherein X_{96} is leucine or tryptophan.
- 39. The amino acid sequence according to claim 38 wherein X_{96} is leucine.
- 40. The amino acid sequence according to claim 39 wherein X₉₅ is glutamine, and X₉₆ is tryptophan.
 - 41. The amino acid sequence according to claim 40 wherein X₉₃ is valine.
 - 42. The amino acid sequence according to claim 41 wherein asparagine is bound to the amino terminal of X_1 .
- 43. An amino acid sequence selected from the amino acid sequences listed in Figures 1-A through 1-O.

- The amino acid sequence according to claim 25 wherein the sequence is selected from the group consisting of FHENFYDWFVRQVS, DYKDVTFTSAVFHENFYDWFVRQVSKK, GRVDWLQRNANFYDWFVAELG and APTFYAWFNQQT.
- 5 45. The amino acid sequence according to claim 25 wherein the sequence comprises FHENFYDWFVRQVS.
 - 46. The amino acid sequence according to claim 25 wherein the sequence is selected from the group consisting of

FHENFYDWFVRQVAKK-NH₂

FHENFYDWFVRAVSKK-NH₂

FHENFYDWFVAQVSKK-NH₂

FHENFYDWFARQVSKK-NH₂

FHEAFYDWFVRQVSKK-NH₂

15 FHANFYDWFVRQVSKK-NH₂ FAENFYDWFVRQVSKK-NH₂

AHENFYDWFVRQVSKK-NH₂

fhenfydwfvrqvskk

EFHENFYDWFVRQVSEE

20 FHENFYGWFVRQVSKK

HETFYSMIRSLAK SDGFYNAIELLS

SLNFYDALQLLAKK

HDPFYSMMKSLLK

25 NSFYEALRMLSSK

HPTSKEIYAKLLK

HPSTNQMLMKLFK

HPPLSELKLFLIKK

HAPLSVLVQALLKK

30 HPSLSDMRWILLK

WSDFYSYFQGLD

D117-Dap(D117)

SSNFYQALMLLS

D117-Dap(CO-CH₂-O-NH₂)

35 HENFYGWFVRQVSKK

D117-Lys(D117)

	D117-b-Ala-Lys(D117) D117-b-Ala-Dap(b-Ala-D117) D117-Gly-Lys(Gly-D117)
	D117-b-Ala-Lys(b-Ala-D117)
5	D117-Dab(D117)
	D117-Orn(D117)
	D117-Dap(b-Ala-D117)
	D117-b-Ala-Orn(b-Ala-D117)
	$\underline{1}$ -(Thia-b-Ala-D117) ₂
10	FHENFYDWFVRQVS
	FHENFYDWFVRQVSK
	FHENFYDWFVQVSK
	FHENFYDWFVVSK
	FHENFYDWFVSK
15	FHENFYDWFVK
	FYDWF-NH ₂
	FYDWFKK-NH ₂
	AFYDWFAKK-NH ₂
	AAAAFYDWFAAAAAKK-NH ₂
20	$(D117)_2 - \underline{12}$
	$(Cys-Gly-D117)_2$
	Cys-Gly-D117
	(D117) ₂ - <u>14</u>
	LDALDRLMRYFEERPSL-NH ₂
25	PLAELWAYFEHSEQGRSSAH-NH ₂
	GRVDWLQRNANFYDWFVACLG-NH ₂
	NGVERAGTGDNFYDWFVAQLH-NH2
	EHWNTVDPFYFTLFEWLRESG-NH ₂
- ^	EHWNTVDPFYQYFSELLRESG-NH ₂ QSDSGTVHDRFYGWFRDTWAS-NH ₂
30	
	AFYDWFA NH
	AFYDWFA-NH
	${ m AFYDWF-NH_2}$ ${ m FYDWDA-NH_2}$
25	Ac-FYDWF-NH ₂
35	Lig-FHENFYDWFVRQVSKK
	Lig-GGGFHENFYDWFVRQVSKK
	FHENFYDWFVRQVSKKGGG-Lig
	Lig-CAWPTYWNCG
40	ACAWPTYWNCG-Lig
40	ACAWPTYWNCGGGG-Lig
	Lig-SDGFYNAIELLS
	SDGFYNAIELLS-Lig
	<u> </u>

	SDGFYNAIELLSGGG-Lig
	KHLCVLEELFWGASLFGYCSGKK-Lig
	AFYDWFAKK-Lig
	AFYEWFAKK-NH ₂
5	AFYGWFAKK-NH ₂
	$AFYKWFAKK-NH_2$
	(SDGFYNAIELLS-Lig) ₂ - <u>14</u>
	(AFYDWFAKK-Lig) ₂ - <u>14</u>
	FHENAYDWFVRQVSKK
10	FHENFADWFVRQVSKK
	FHENFYAWFVRQVSKK
	FHENFYDAFVRQVSKK
	FHENFTDWAVRQVSKK
	FQSLLEELVWGAPLFRYGTG
15	PLCVLEELFWGASLFGQCSG
	QLEEEWAGVQCEVYGRECPS
	$Cys-(Gly)_2-D117$
	$(Cys-(Gly)_2-D117)_2$
	(S210)- <u>14</u> -(S212)
20	(S131)- <u>14</u> -(S212)
	$(S205)_2$ -14
	$(S204)_2$ -14
	(S131)- <u>14</u> -(S210)
	RVDWLQRNANFYDWFVAELG
25	VDWLQRNANFYDWFVAELG
	DWLQRNANFYDWFVAELG
	WLQRNANFYDWFVAELG
	LQRNANFYDWFVAELG
	QRNANFYDWFVAELG
30	RNANFYDWFVAELG
	NANFYDWFVAELG
	ANFYDWFVAELG
	NFYDWFVAELG
	GRVDWLQRNANFYDWFVAELG-Lig
35	Lig-GRVDWLQRNANFYDWFVAELG
	(S208)- <u>14</u> -(S131)
	(S208)- <u>14</u> -(S209)
	GRVDWLQRNANFYDWFVAEL
4.0	GRVDWLQRNANFYDWFVAE
40	GRVDWLQRNANFYDWFV
	GRVDWLQRNANFYDWFV
	14-(SDGFYNAIELLS-Lig) ₂
	(GRVDWLQRNANFYDWFVAELG)- <u>14</u>

	14-(GRVDWLQRNANFYDWFVAE LG)
	(SDGFYNAIELLSGGG) ₂ - <u>14</u>
	H-Acy-CLEE-w-GASL-Tic-QCSG-NH ₂
	RWPNFYGYFESLLTHFS-NH ₂
5	HYNAFYEYFQVLLAETW-NH ₂
	EGWDFYSYFSGLLASVT-NH ₂
	LDRQFYRYFQDLLVGFM-NH ₂
	WGRSFYRYFETLLAQGI-NH ₂
	PLCFLQELFGGASLGGYCSG-NH ₂
10	WLEQERAWIWCEIQGSGCRA-NH ₂
	IQGWEPFYGWFDDVVAQMFEE-NH ₂
	TGHRLGLDEQFYWWFRDALSG-NH ₂
	H-Abu-CLEE-w-GASL-Tic-QCSG-NH ₂
	14-(Dap-CAWPTYWNCG) ₂
15	RDHypFYDWFDDi-NH2
	S131- <u>14</u> -S209
	S294- <u>14</u> -S210
	S295- <u>14</u> -S210
	S294- <u>14</u> -204
20	S295- <u>14</u> -S204
	GFREGQRWYWFVAQVT-NH ₂
	VASGHVLHGQFYRWFVDQFALEE-NH ₂
	VGDFCVSHDCFYGWFLRESMQ-NH ₂
	DLRVLCELFGGAYVLGYCSE-NH ₂
25	HLSVGEELSWWVALLGQWAR-NH ₂
	APVSTEELRWGALLFGQWAG-NH ₂
	ALEEEWAWVQVRSIRSGLPL-NH ₂
	WLEHEWAQIQCELYGRGCTY-NH ₂
20	AAVHEQFYDWFADQYEE-NH ₂
30	QAPSNFYDWFVREWDEE-NH ₂
	QSFYDYIEELLGGEWKK-NH ₂
	DPFYQGLWEWLRESGEE-NH ₂
	$(\$204)_2 - 7$
25	$(\$204)_2 - 9$
35	$(\$204)_2 - \underline{12}$
	(\$204) ₂ - <u>13</u>
	DWLQRNANFYDWFVAEL-Lig
	Lig-DWLQRNANFYDWFVAEL (S209) ₂ -9
40	$(8210)_2$ -9
TV	LigKHLCVLEELFWGASLFGYCSGKKKK
	KHLCVLEELFWGASLFGYCSGKKKK-Lig
	(\$294) ₂ -14
	(Sas 1/2 <u>1.1</u>

(S295)₂-<u>14</u>
S-D-G-F-Y-N-A-Acy-E-L-L-S
S-G-P-F-Y-E-E-Acy-E-L-L-W-Aib
G-G-S-F-Y-D-D-Acy-E-Aib-L-W-Aib
N-Aib-P-F-Y-D-E-Acy-D-E-Cha-W-Aib
GRVDWLQRNANFYDWFVAEAcyG-NH₂
and wherein underlined numbers represent a linker as defined in Table 18.

- 47. An amino acid sequence which specifically binds IR such that binding to IGF-1R is at or below background and wherein said amino acid sequence comprises $X_1X_2X_3X_4X_5$ wherein X_1 , X_2 , and X_5 are selected from the group consisting of phenylalanine and tyrosine, X_3 is selected from the group consisting of aspartic acid, glutamic acid, glycine and serine, and X_4 is selected from group consisting of tryptohpan, tyrosine and phyenylalanine.
- 48. A method of modulating insulin activity in mammalian cells, said method comprising administering to said cells an amino acid sequence which binds IR and comprises the sequence of amino acids $X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$ wherein X_6 and X_7 are aromatic amino acids or glutamine, X_8 , X_9 , X_{11} and X_{12} may be any amino acid, X_{10} and X_{13} are hydrophobic amino acids.
- 49. The method according to claim 48 wherein X₆ and X₇ are selected from group consisting of phenylalanine and tyrosine, and X₁₀ and X₁₃ are selected from group consisting of leucine, isoleucine, tryptophan, phenylalanine methionine and valine.
 - 50. The method according to claim 48 wherein X_6 is phenylalanine and X_7 is tyrosine.
- 25 51. The method according to claim 50 wherein X_{10} is isoleucine.
 - 52. The method according to claim 50 wherein X_{10} is leucine.

- 53. The method according to claim 50 wherein X_{13} is leucine.
- 54. The method according to claim 50 wherein X_9 is tyrosine and X_{10} is phenylalanine.
- 55. The method according to claim 50 wherein the amino acid sequence is selected from FYX₈X₉LX₁₁X₁₂L, FYX₈X₉IX₁₁X₁₂L and FYX₈YFX₁₁X₁₂L.
 - 56. The method according to claim 55 wherein the amino acid sequence comprises FYX₈X₉LX₁₁X₁₂L.
 - 57. The method according to claim 55 wherein the amino acid sequence comprises FYX₈ YFX₁₁X₁₂L.
- The method according to claim 48 wherein the amino acid sequence $X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$ further comprises amino acids X_{98} and X_{99} at the amino terminal end and X_{100} at the carboxy terminal end to form $X_{98}X_{99}X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{100}$ and wherein X_{98} is optionally aspartic acid and X_{99} is independently an amino acid selected from the group consisting of glycine, glutamine and proline, and X_{100} is a hydrophobic amino acid.
 - 59. The method according to claim 58 wherein X_{100} is an aliphatic amino acid.
 - 60. The method according to claim 59 wherein X_{100} is leucine.
- The method according to claim 48 wherein the amino acid sequence binds to the insulin receptor with an affinity of at least about 10⁻⁵ M.

- 62. The method according to claim 61 wherein the affinity is between about 10⁻⁷ M.
- 63. The method according to claim 48 wherein the amino acid sequence comprises DYKDFYDAIDQLVRGSARAGGTRD or KDRAFYNGLRDLVGAVYGAWD.
 - 64. The method according to claim 48 wherein the amino acid sequence is selected from the group of amino acid sequences listed in Figures 2A through 2P.
- An amino acid sequence comprising X₆X₇X₈X₉X₁₀X₁₁X₁₂X₁₃ wherein X₆
 and X₇ are aromatic amino acids or glutamine, X₈, X₉, X₁₁ and X₁₂ may be any amino acid, X₁₀ and X₁₃ are hydrophobic amino acids and wherein said amino acid sequence binds to IGF-1R.
 - 66. The amino acid sequence according to claim 65 wherein the binding occurs at an affinity (K_d) of at least about 10^{-5} M.
- The amino acid sequence according to claim 66 wherein the binding occurs at an affinity (K_d) of at least about 10^{-7} M.
 - 68. The amino acid sequence according to claim 65 wherein X_6 and X_7 are phenylalanine or tyrosine, and X_{10} and X_{13} are leucine, isoleucine, tryptophan, phenylalanine or methionine.
- The amino acid sequence according to claim 68 wherein X_6 is phenylalanine and X_7 is tyrosine.

- 70. The amino acid sequence according to claim 68 wherein X_{10} is isoleucine.
- 71. The amino acid sequence according to claim 68 wherein X_{10} is leucine.
- 72. The amino acid sequence according to claim 69 wherein X_{13} is leucine.
- 73. The amino acid sequence according to claim 69 wherein X₉ is tyrosine and X₁₀ is phenylalanine.
 - 74. The amino acid sequence according to claim 68 wherein the amino acid sequence comprises an amino acid sequence selected from FYX₈X₉LX₁₁X₁₂L, FYX₈X₉IX₁₁X₁₂L and FYX₈YFX₁₁X₁₂L.
- 75. The amino acid sequence according to claim 74 wherein the amino acid sequence comprises FYX₈X₉IX₁₁X₁₂L.
 - 76. The amino acid sequence according to claim 74 wherein the amino acid sequence comprises $FYX_8X_9LX_{11}X_{12}L$.
 - 77. The amino acid sequence according to claim 74 wherein the amino acid sequence is $FYX_8YFX_{11}X_{12}L$.
- The amino acid sequence according to claim 65 wherein the amino acid sequence $X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$ further comprises amino acids X_{98} and X_{99} at the amino terminal end and X_{100} at the carboxy terminal end to form $X_{98}X_{99}X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{100}$ and wherein X_{98} is optionally aspartic acid and X_{99} is independently an amino acid selected from the group consisting of glycine, glutamine and proline, and X_{100} is a hydrophobic amino acid.

- 79. The amino acid sequence according to claim 78 wherein X_{100} is an aliphatic amino acid.
- 80. The amino acid sequence according to claim 79 wherein X_{100} is leucine.
- 81. The amino acid sequence according to claim 68 wherein the amino acid sequence comprises DYKDFYDAIDQLVRGSARAGGTRD or KDRAFYNGLRDLVGAVYGAWDKK.
 - 82. The sequence according to claim 81 wherein the amino acid sequence comprises DYKDFYDAIDQLVRGSARAGGTRD.
- 83. An amino acid sequence comprising an amino acid sequence selected from the group consisting of amino sequences listed in Figures 2A through 2P.
 - 84. An amino acid sequence comprising a sequence selected from the group consisting of
- SFYEAIHQLLGV,
 NSFYEALRMLSS,
 SLNFYDALQLLA,
 SSNFYQALMLLS,
 SDGFYNAIELLS,
 HETFYSMIRSLA,
 HDPFYSMMKSLL and
 WSDFYSYFQGLD.

85. The amino acid sequence according to claim 65 wherein the sequence comprises the amino acid sequence $X_{115}X_{116}X_{117}X_{118}FYX_8YFX_{11}X_{12}LX_{119}X_{120}X_{121}X_{122}$ wherein X_{115} is selected from the group consisting of trytophan, glycine, aspartic acid, glutamic acid 5 and arginine, X_{116} is selected from the group consisting of aspartic acid, histidine, glycine and asparagine, X_{117} and X_{118} are selected from the group consisting of glycine, aspartic acid, glutamic acid, asparagine, and alanine, X₈ is selected from the group consisting of arginine, glycine, glutamic acid and serine, X_{11} is selected from the group consisting of glutamic acid, 10 asparagine, glutamine and tryptophan, X₁₂ is selected from the group consisting of aspartic acid, glutamic acid, glycine, lysine, and glutamine, X_{119} is selected from the group consisting of glutamic acid, glycine, glutamine, aspartic acid and alanine, X_{120} is selected from the group consisting of glutamic acid, aspartic acid, glycine and glutamine, X_{121} is 15 selected from the group consisting of tryptophan, tyrosine, glutamic acid, phenylalanine, histidine and aspartic acid, and X_{122} is selected from the

86. The amino acid sequence according to claim 85 wherein X_{115} is tryptophan, X_{117} is selected from glycine, aspartic acid, glutamic acid and asparagine; X_{118} is selected from glycine, aspartic acid, glutamic acid and alanine; X_{11} , X_{119} , X_{120} , and X_{122} are glutamic acid; X_{12} is aspartic acid, and X_{121} is tryptophan or tyrosine.

group consisting of glutamic acid, aspartic acid, and glycine.

An amino acid sequence comprising X₆X₇X₈X₉X₁₀X₁₁X₁₂X₁₃ wherein X₆ and X₇ are aromatic amino acids or glutamine, X₈, X₉, X₁₁ and X₁₂ may be any amino acid, X₁₀ and X₁₃ are hydrophobic amino acids and wherein said amino acid sequence binds to IR such that binding to IGF-1R is at or below background.

- A method of binding to Site 1 of IR from mammalian cells, said method comprising contacting IR with an amino acid sequence which binds IR and comprises the sequence of $X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}$ wherein X_{14} , X_{17} , and X_{18} are hydrophobic amino acids, X_{15} , X_{16} , and X_{19} are any amino acid, and X_{20} and X_{21} are aromatic amino acids.
- 89. The method according to claim 88 wherein X₁₄ and X₁₇ are selected from the group consisting of leucine, isoleucine and valine; X₂₀ is selected from group consisting of tyrosine and histidine; and X₂₁ is selected from group consisting of phenylalanine and tyrosine.
- 10 90. The method according to claim 89 wherein X_{14} and X_{17} are leucine.
 - 91. The method according to claim 89 wherein X_{14} is leucine.
 - 92. The method according to claim 89 wherein X_{17} is leucine.
 - 93. The method according to claim 89 wherein X_{20} is tyrosine.
 - 94. The method according to claim 89 wherein X_{21} is phenylalanine.
- 15 95. The method according to claim 90 wherein X_{15} is a large amino acid.
 - 96. The method according to claim 89 wherein said amino acid sequence further comprises an amino acid extension comprising $X_{101}X_{102}X_{103}$ wherein X_{103} is bound to X_{14} at the amino terminus and X_{101} and X_{102} are polar amino acids and X_{103} is a hydrophobic amino acid.
- 20 97. The method according to claim 96 wherein X_{101} and X_{102} are independently aspartic acid or glutamic acid and X_{103} is leucine, isoleucine or valine.

- 98. A method of binding to Site 1 of IGF-1R from mammalian cells, said method comprising contacting IGF-1R with an amino acid sequence which binds IR and comprises the sequence of $X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}$ wherein X_{14} , X_{17} , and X_{18} are hydrophobic amino acids, X_{15} , X_{16} , and X_{19} are any amino acid, and X_{20} and X_{21} are aromatic amino acids.
- 99. The method according to claim 98 wherein X₁₄ and X₁₇ are selected from the group consisting of leucine, isoleucine and valine; X₁₈ is an aromatic amino acid; X₂₀ is selected from group consisting of tyrosine and histidine; and X₂₁ is selected from group consisting of phenylalanine and tyrosine.
- 10 100. The method according to claim 98 wherein the amino acid sequence comprises a sequence selected from the sequences in Figures 3A through 3D.
- 101. An amino acid sequence which binds Site 1 of IR from mammalian cells, said sequence comprising X₁₄X₁₅X₁₆X₁₇X₁₈X₁₉X₂₀X₂₁ wherein X₁₄, X₁₇, and X₁₈ are hydrophobic amino acids, X₁₅, X₁₆, and X₁₉ are any amino acid, and X₂₀ and X₂₁ are aromatic amino acids.
 - 102. The amino acid sequence according to claim 101 wherein X_{14} and X_{17} are selected from the group consisting of leucine, isoleucine and valine; X_{20} is selected from group consisting of phenylalanine and tyrosine.
- 20 103. The amino acid sequence according to claim 102 wherein X_{14} and X_{17} are leucine.
 - 104. The amino acid sequence according to claim 102 wherein X_{14} is leucine.
 - 105. The amino acid sequence according to claim 102 wherein X_{17} is leucine.

- 106. The amino acid sequence according to claim 102 wherein amino acid X_{18} is tryptophan.
- 107. The amino acid sequence according to claim 103 wherein X_{20} is tyrosine.
- The amino acid sequence according to claim 107 wherein X₂₁ is
 phenylalanine.
 - 109. The amino acid sequence according to claim 103 wherein X_{15} is a large amino acid.
 - 110. The amino acid sequence according to claim 101 wherein at least one amino acid is a D-amino acid.
- 10 111. The amino acid sequence according to claim 65 wherein at least one amino acid is a D-amino acid.
- 112. The amino acid sequence according to claim 102 wherein said amino acid sequence further comprises an amino acid extension comprising $X_{101}X_{102}X_{103}$ wherein X_{103} is bound to X_{14} at the amino terminus and X_{101} and X_{102} are polar amino acids and X_{103} is a hydrophobic amino acid.
 - 113. The amino acid sequence according to claim 112 wherein X_{101} and X_{102} are independently aspartic acid or glutamic acid and X_{103} is leucine, isoleucine or valine.

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- 114. An amino acid sequence which binds Site 1 of IGF-1R from mammalian cells such that binding to IR is at or below background, said sequence comprising $X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}$ wherein X_{14} , X_{17} , and X_{18} are hydrophobic amino acids, X_{15} , X_{16} , and X_{19} are any amino acid, and X_{20} and X_{21} are aromatic amino acids.
- 115. The amino acid sequence according to claim 114 wherein X₁₄ and X₁₇ are selected from the group consisting of leucine, isoleucine and valine; X₁₈ is an aromatic amino acid; X₂₀ is selected from group consisting of tyrosine and histidine; and X₂₁ is selected from group consisting of phenylalanine and tyrosine.
- 116. A method of binding to Site 2 of IR from mammalian cells, said method comprising contacting said cells with an amino acid sequence comprising $X_{22}X_{23}X_{24}X_{25}X_{26}X_{27}X_{28}X_{29}X_{30}X_{31}X_{32}X_{33}X_{34}X_{35}X_{36}X_{37}X_{38}X_{39}X_{40}X_{41}$ wherein X_{22} , X_{25} , X_{26} , X_{28} , X_{29} , X_{30} , X_{33} , X_{34} , X_{35} , X_{37} , X_{38} , X_{40} and X_{41} are any amino acid; X_{23} is any hydrophobic amino acid; X_{27} is a polar amino acid; X_{31} is an aromatic amino acid; X_{32} is a small amino acid; and wherein at least one cysteine is located at positions X_{24} through X_{27} and one at X_{39} or X_{40} .
 - 117. The method according to claim 116 wherein X_{24} and X_{39} are cysteines.
- 20 118. The method according to claim 117 wherein X₂₃ is selected from leucine, isoleucine, methionine and valine; X₂₇ is selected from glutamic acid, aspartic acid, asparagine, and glutamine; X₃₁ is tryptophan, X₃₂ is glycine; and X₃₆ is any aromatic amino acid.
- 119. The method according to claim 118 wherein the binding to IR occurs at an affinity (K_d) of at least about 10⁻⁵ M.

- 120. The method according to claim 116 wherein X_{23} is leucine, X_{27} is glutamic acid, X_{31} is tryptophan, and X_{32} is glycine.
- 121. The method according to claim 116 wherein the amino acid sequence is HLCVLEELFWGASLFGYCSG.
- An amino acid sequence which binds IR, said amino acid sequence comprising

 X₂₂X₂₃X₂₄X₂₅X₂₆X₂₇X₂₈X₂₉X₃₀X₃₁X₃₂X₃₃X₃₄X₃₅X₃₆X₃₇X₃₈X₃₉X₄₀X₄₁

 wherein X₂₂, X₂₅, X₂₆, X₂₈, X₂₉, X₃₀, X₃₃, X₃₄, X₃₅, X₃₇, X₃₈, X₄₀ and X₄₁ are any amino acid, X₂₃ is any hydrophobic amino acid, X₂₇ is a polar amino acid; X₃₁ is an aromatic amino acid; X₃₂ is a small amino acid, and wherein at least one cysteine is located at positions X₂₄ through X₂₇ and one at X₃₉ or X₄₀.
 - 123. The amino acid sequence according to claim 122 wherein X_{24} and X_{39} are cysteines.
- 15 124. The amino acid sequence according to claim 123 wherein X₂₃ is selected from methionine, valine, and leucine; X₂₇ is selected from glutamic acid, alanine, glycine, glutamine, aspartic acid and valine; X₃₁ and X₃₂ are small amino acids; and X₃₆ is an aromatic amino acid.
- The amino acid sequence according to claim 122 wherein the binding to IR occurs at an affinity (K_d) of at least about 10⁻⁵ M.
 - 126. The amino acid sequence according to claim 124 wherein X_{23} is leucine, X_{27} is glutamic acid, X_{31} is tryptophan, and X_{32} is glycine.

- 127. The amino acid sequence according to claim 122 wherein the amino acid sequence is HLCVLEELFWGASLFGYCSG.
- 128. A method of modulating insulin activity in mammalian cells, said method comprising administering to said cells an amino acid sequence which binds

 IR and comprises the sequence X₄₂ X₄₃ X₄₄ X₄₅ X₄₆ X₄₇ X₄₈ X₄₉ X₅₀ X₅₁ X₅₂ X₅₃ X₅₄ X₅₅ X₅₆X₅₇X₅₈X₅₉ X₆₀ X₆₁ wherein X₄₂, X₄₃, X₄₄, X₄₅, X₅₃, X₅₅, X₅₆, X₅₈, X₆₀ and X₆₁ are any amino acid; X₄₃, X₄₆, X₄₉, X₅₀ and X₅₄ are hydrophobic amino acids; X₄₇ and X₅₉ are cysteines; X₄₈ is a polar amino acid; X₅₁, X₅₂ and X₅₇ are small amino acids.
- 10 129. The method according to claim 128 wherein X_{43} and X_{46} are leucine; X_{48} is selected from the group consisting of aspartic acid and glutamic acid; X_{50} is phenylalanine or tyrosine; and X_{51} , X_{52} and X_{57} are glycine.
 - 130. The method according to claim 129 wherein X_{48} is glutamic acid and X_{50} is a phenylalanine.
- 15 131. The method according to claim 130 wherein the amino acid sequence is X_{42} X_{43} X_{44} X_{45} LCE X_{49} FGG X_{53} X_{54} X_{55} X_{56} GX₅₈C X_{60} X_{61} .
 - 132. The method according the claim 131 wherein the amino acid sequence comprises DLRVLCELFGGAYVLGYCSE or DLRVLCELFGGAYVRGYCSE.
- 20 133. The method according to claim 128 wherein the binding to IR occurs at an affinity (K_d) of at least about 10^{-5} M.

- 134. An amino acid sequence which binds IR, said amino acid sequence comprising X₄₂ X₄₃ X₄₄ X₄₅ X₄₆ X₄₇ X₄₈ X₄₉ X₅₀ X₅₁ X₅₂ X₅₃ X₅₄ X₅₅ X₅₆X₅₇X₅₈X₅₉ X₆₀ X₆₁ wherein X₄₂, X₄₃, X₄₄, X₄₅, X₅₃, X₅₅, X₆₀ and X₆₁ are any amino acid; X₄₃, X₄₆, X₄₉, X₅₀ and X₅₄ are hydrophobic amino acids; X₄₇ and X₅₉ are cysteines; X₄₈ is a polar amino acid; and X₅₁ ,X₅₂ and X₅₇ are small amino acids.
- 135. The amino acid sequence according to claim 134 wherein X_{43} and X_{46} are leucine; X_{48} is selected from the group consisting of aspartic acid and glutamic acid; X_{50} is phenylalanine or tyrosine; and X_{51} , X_{52} and X_{57} are glycine.
- 136. The amino acid sequence according to claim 135 wherein X_{48} is glutamic acid and X_{50} is phenylalanine.
- 137. The amino acid sequence according to claim 136 wherein the amino acid sequence comprises X₄₃ X₄₄ X₄₅ LCE X₄₉ FGG X₅₃ X₅₄ X₅₅ X₅₆ G X₅₈ C X₆₀
 15 X₆₁.
 - 138. The amino acid sequence according to claim 137 wherein an amino acid sequence comprises DLRVLCELFGGAYVLGYCSE or DLRVLCELFGGAYVRGYCSE
- 20 comprising administering to said cells an amino acid sequence comprising $X_{62} X_{63} X_{64} X_{65} X_{66} X_{67} X_{68} X_{69} X_{70} X_{71} X_{72} X_{73} X_{74} X_{75} X_{76} X_{77} X_{78} X_{79} X_{80} X_{81}$ wherein X_{62} , X_{65} , X_{66} , X_{68} , X_{69} , X_{71} , X_{73} , X_{76} , X_{77} , X_{78} , X_{80} and X_{81} are any amino acid; X_{63} , X_{70} , and X_{74} are hydrophobic amino acids; X_{64} is a polar amino acid; X_{67} and X_{75} are aromatic amino acids; and X_{72} and X_{79} are cysteines.

- 140. The method according to claim 139 wherein X_{63} is selected from the group consisting of leucine, isoleucine, methionine and valine; X_{70} and X_{74} are selected from group consisting of valine, isoleucine, leucine and methionine; X_{64} is selected from group consisting of aspartic acid and glutamic acid; X_{67} is tryptophan; and X_{75} is selected from group consisting of tyrosine and tryptophan.
- 141. The method according to claim 140 wherein X_{66} is glutamic acid.
- 142. The method according to claim 141 wherein X_{63} is leucine.
- 143. The method according to claim 140 wherein X_{74} is valine.
- 10 144. The method according to claim 141 wherein X_{64} is a glutamic acid.
 - 145. The method according to claim 141 wherein X_{75} is a tyrosine.
 - 146. The method accord to claim 140 wherein the amino acid sequence comprises WLDQEWAWVQCEVYGRGCPS.
- 147. An amino acid sequence which binds IR, said amino acid sequence

 comprising X₆₂ X₆₃ X₆₄ X₆₅ X₆₆ X₆₇ X₆₈ X₆₉ X₇₀ X₇₁ X₇₂ X₇₃ X₇₄ X₇₅ X₇₆ X₇₇

 X₇₈ X₇₉ X₈₀ X₈₁ wherein X₆₂, X₆₅, X₆₆ X₆₈, X₆₉, X₇₁, X₇₃, X₇₆, X₇₇, X₇₈, X₈₀

 and X₈₁ are any amino acid; X₆₃, X₇₀, and X₇₄ are hydrophobic amino acids;

 X₆₄ is a polar amino acid; X₆₇ and X₇₅ are aromatic amino acids; and X₇₂ and

 X₇₉ are cysteines.

- 148. The amino acid sequence according to claim 147 wherein X_{63} is selected from the group consisting of leucine, isoleucine, methionine and valine; X_{70} and X_{74} are selected from group consisting of valine, isoleucine, leucine and methionine; X_{64} is selected from group consisting of aspartic acid and glutamic acid; X_{67} is tryptophan; and X_{75} is selected from group consisting of tyrosine and tryptophan.
- 149. The amino acid sequence according to claim 148 wherein X_{66} is glutamic acid.
- 150. The amino acid sequence according to claim 149 wherein X_{63} is leucine.
- 10 151. The amino acid sequence according to claim 148 wherein X_{74} is valine.
 - 152. The amino acid sequence according to claim 149 wherein X_{64} is glutamic acid.
 - 153. The amino acid sequence according to claim 148 wherein X_{75} is a tyrosine.
- 154. The amino acid sequence accord to claim 148 wherein the amino acid sequence comprises WLDQEWAWVQCEVYGRGCPS.
 - 155. The amino acid sequence according to claim 148 wherein the affinity (K_d) of binding to IR is at least 10^{-5} M.
 - 156. The amino acid sequence according to claim 148 wherein the amino acid sequence comprises a sequence selected from the sequences of Figures 6A-6F.

- 157. A method of modulating insulin activity in mammalian cells, said method comprising administering to said cells an amino acid sequence which binds IR and comprises HX₈₂X₈₃X₈₄X₈₅X₈₆X₈₇X₈₈X₈₉X₉₀X₉₁X₉₂ herein X₈₂ is proline or alanine; X₈₃ is a small amino acid; X₈₄ is selected from the group consisting of leucine, serine and threonine; X₈₅ is a polar amino acid; X₈₆ is any amino acid; X₈₇ is an aliphatic amino acid; X₈₈, X₈₉, X₉₀ is any amino acid; and X₉₁ and X₉₂ are aliphatic amino acids.
- 158. The method according to claim 157 wherein X₈₂ is proline; X₈₃ is selected from the group consisting of proline, serine and threonine; X₈₄ is leucine; X₈₅ is selected from the group consisting of glutamic acid, serine, lysine and asparagine; X₈₆ is a polar amino acid; X₈₇ is selected from the group consisting of leucine, methionine and isoleucine; and X₉₁ and X₉₂ are leucines.
 - 159. The method according to claim 158 wherein X₈₃ is proline.
- 15 160. The method according to claim 158 wherein X_{85} is serine.
 - 161. The method according to claim 158 wherein X_{86} is selected from the group consisting of histidine, glutamic acid, aspartic acid and glutamine.
 - 162. The method according to claim 158 wherein X_{87} is leucine.
 - 163. The method according to claim 158 wherein X_{92} is phenylalanine.
- 20 164. The method according to claim 160 wherein the amino acid sequence is HPPLSX₈₆ LX₈₈ X₈₉ X₉₀ LL.

- 165. The method according to claim 158 wherein the amino acid sequence is selected from the group consisting of HPPLEHLKAFLL, HPPLSELKLFLI, HPSLSDMRWILL, HPTSKEIYAKLL, HPTSKEIYAKLL, HPSTNQMLMKLF and HAPLSVLQALL.
- 5 166. An amino acid sequence which binds IR, said amino acid sequence comprising HX₈₂X₈₃X₈₄X₈₅X₈₆X₈₇X₈₈X₈₉X₉₀X₉₁X₉₂ herein X₈₂ is proline or alanine; X₈₃ is a small amino acid; X₈₄ is selected from the group consisting of leucine, serine and threonine; X₈₅ is a polar amino acid; X₈₆ is any amino acid; X₈₇ is an aliphatic amino acid; X₈₈, X₈₉, X₉₀ is any amino acid; and X₉₁ and X₉₂ are aliphatic amino acids.
 - 167. The amino acid sequence according to claim 166 wherein X_{82} is proline; X_{83} is selected from the group consisting of proline, serine and threonine; X_{84} is leucine; X_{85} is selected from the group consisting of glutamic acid, serine, lysine and asparagine; X_{86} is a polar amino acid; X_{87} is selected from the group consisting of leucine, methionine and isoleucine; and X_{91} and X_{92} are leucines.
 - 168. The amino acid sequence according to claim 167 wherein X_{83} is proline.
 - 169. The amino acid sequence according to claim 167 wherein X_{85} is serine.
- The amino acid sequence according to claim 167 wherein X₈₆ is selected from the group consisting of histidine, glutamic acid, aspartic acid and glutamine.
 - 171. The amino acid sequence according to claim 167 wherein X_{87} is leucine.

- 172. The amino acid sequence according to claim 167 wherein X_{92} is phenylalanine.
- 173. The amino acid sequence according to claim 169 wherein the amino acid sequence is HPPLSX₈₆ LX₈₈ X₈₉ X₉₀ LL.
- The amino acid sequence according to claim 167 wherein the amino acid sequence is selected from the group consisting of HPPLEHLKAFLL, HPPLSELKLFLI, HPSLSDMRWILL, HPTSKEIYAKLL, HPSTNQMLMKLF and HAPLSVLQALL.
- 175. A method modulating insulin activity in mammalian cells, said method

 10 comprising administering to said cells an amino acid sequence comprising
 an amino acid sequence of $X_{104}X_{105}X_{106}X_{107}X_{108}X_{109}X_{110}X_{111}X_{112}X_{113}X_{114}$ wherein at least one of the amino acids of X_{106} through X_{111} are tryptophan;
 wherein X_{104} and X_{114} are both small amino acids; wherein X_{105} is any
 amino acid; and wherein at least one of X_{104} , X_{105} , X_{106} and one of X_{112} X_{113} 15 X_{114} are cysteine residues.
 - 176. The method according to claim 175 wherein at least two of the amino acids of X_{106} through X_{111} are tryptophan which are separated from each other by at least two amino acids.
- 177. The method according to claim 176 wherein the separating amino acids are selected from the group consisting of proline, threonine and tyrosine.
 - 178. The method according to claim 177 wherein the amino acid sequence comprises WPTYW.

- 179. The method according to claim 178 wherein X_{105} and X_{113} are cysteine residues.
- 180. The method according to claim 178 wherein X_{104} and X_{114} are selected from the group consisting of alanine and glycine.
- 5 181. The method according to claim 180 wherein X_{104} is alanine and X_{114} is glycine.
 - 182. The method according to claim 181 wherein X_{105} is valine.
 - 183. The method according to claim 182 wherein X_{112} is asparagine.
- 184. The method according to claim 198 wherein the affinity (K_d) of binding to IR is at least about 10^{-5} M.
 - 185. A method of modulating insulin activity in mammalian cells, said method comprising administering to said cells an amino acid sequence comprising an amino acid sequence selected from the group listed in Figure 8.
- 186. The method according to claim 185 wherein the sequence comprisesACVWPTYWNCG.
 - 187. An amino acid sequence which binds and IR and comprising an amino acid sequence of $X_{104}X_{105}X_{106}X_{107}X_{108}X_{109}X_{110}X_{111}X_{112}X_{113}X_{114}$ wherein at least one of the amino acids of X_{106} through X_{111} are tryptophan; wherein X_{104} and X_{114} are both small amino acids; wherein X_{105} is any amino acid; and wherein at least one of X_{104} , X_{105} , X_{106} and one of X_{112} X_{113} X_{114} are cysteine residues.

- 188. The amino acid sequence according to claim 187 wherein at least two of the amino acids of X_{106} through X_{111} are tryptophan which are separated from each other by at least two amino acids.
- The amino acid sequence according to claim 188 wherein the separating
 amino acids are selected from the group consisting of proline, threonine and tyrosine.
 - 190. The amino acid sequence according to claim 189 wherein the amino acid sequence comprises WPTYW.
- 191. The amino acid sequence according to claim 190 wherein X_{105} and X_{113} are cysteine residues.
 - 192. The amino acid sequence according to claim 190 wherein X_{104} and X_{114} are selected from the group consisting of alanine and glycine.
 - 193. The amino acid sequence according to claim 190 wherein X_{104} is alanine and X_{114} is glycine.
- 15 194. The amino acid sequence according to claim 193 wherein X_{105} is valine.
 - 195. The amino acid sequence according to claim 194 wherein X_{112} is asparagine.
 - 196. The amino acid sequence according to claim 202 wherein the affinity (K_d) of binding to IR is at least about 10⁻⁵ M.
- 197. An amino acid sequence which binds IR from mammalian cells comprising an amino acid sequence selected from the group listed in Figure 8.

- 198. The amino acid sequence according to claim 197 comprising ACVWPTYWNCG.
- 199. A method of providing insulin agonist activity to mammalian cells, said method comprising administering to said cells an amino acid sequence comprising DYKDLCQSWGVRIGWLAGLCPKK.
- 200. A method of modulating insulin activity in mammalian cells, said method comprising administering to said cells an amino acid sequence comprising an amino acid sequence selected from the group listed in Figures 9 through 11.
- 10 201. An amino acid sequence comprising DYKDLCQSWGVRIGWLAGLCPKK.
 - 202. An amino acid sequence comprising an amino acid sequence selected from the group listed in Figures 9 through 11.
- 203. An amino acid sequence comprising at least two amino acid sequences which independently bind IR, with the proviso that at least one of the sequences is not insulin or a fragment thereof.
 - 204. The amino acid sequence according to claim 203 wherein the two amino acid sequences bind to Site 1 of IR.
 - 205. The amino acid sequence according to claim 203 wherein one amino acid sequence binds to Site 1, and the other binds to Site 2 of IR.

- 206. The amino acid sequence according to claim 203, wherein at least one of the sequences is selected from the group consisting of $X_1X_2X_3X_4X_5$ wherein X_1 , X_2 , X_4 , and X_5 are aromatic amino acids, and X_3 may be any polar amino acid; $X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$ wherein X_6 and X_7 are aromatic amino acids or glutamine, X_8 , X_9 , X_{11} and X_{12} may be any amino acid, X_{10} and X_{13} are hydrophobic amino acids; and $X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}$ wherein X_{14} , X_{17} , and X_{18} are hydrophobic amino acids, X_{15} , X_{16} , and X_{19} are any amino acid, and X_{20} and X_{21} are aromatic amino acids.
- The amino acid sequence according to claim 206, wherein at least one of the sequences is $X_1X_2X_3X_4X_5$ wherein X_1 , X_2 , X_4 , and X_5 are aromatic amino acids, and X_3 may be any polar amino acid.
 - 208. The amino acid sequence according to claim 206 wherein at least one of the sequences comprises FYX₃WF.
- The amino acid sequence according to claim 206, wherein at least one of the sequences comprises $X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$ wherein X_6 and X_7 are aromatic amino acids or glutamine, X_8 , X_9 , X_{11} and X_{12} may be any amino acid, X_{10} and X_{13} are hydrophobic amino acids.
 - 210. The amino acid sequence according to claim 209, wherein at least one of the sequences comprises $FYX_8X_9LX_{11}X_{12}L$.
- 20 211. The amino acid sequence according to claim 206, wherein at least one of the sequences comprises $X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}$ wherein X_{14} , X_{17} , and X_{18} are hydrophobic amino acids, X_{15} , X_{16} , and X_{19} are any amino acid, and X_{20} and X_{21} are aromatic amino acids.

- 212. The amino acid sequence according to claim 211 wherein at least one of the sequences comprises LX_{15} , X_{16} , $LLX_{19}YF$.
- 213. The amino acid sequence according to claim 203 wherein at least one of the sequences comprises
- X₂₂X₂₃X₂₄X₂₅X₂₆X₂₇X₂₈X₂₉X₃₀X₃₁X₃₂X₃₃X₃₄X₃₅X₃₆X₃₇X₃₈X₃₉X₄₀X₄₁
 wherein X₂₂, X₂₅, X₂₆, X₂₈, X₂₉, X₃₀, X₃₃, X₃₄, X₃₅, X₃₆, X₃₇, X₃₈, X₄₀, and X₄₁ are any amino acid, X₂₃ is any hydrophobic amino acid; X₂₇ is a polar amino acid; X₃₁ is an aromatic amino acid; X₃₂ is a small amino acid, and wherein at least one cysteine is located at positions X₂₄ through X₂₇ and one
 at X₃₉ or X₄₀; X₄₂ X₄₃ X₄₄ X₄₅ X₄₆ X₄₇ X₄₈ X₄₉ X₅₀ X₅₁ X₅₂ X₅₃ X₅₄ X₅₅ X₅₆X₅₇X₅₈X₅₉ X₆₀ X₆₁ wherein X₄₂, X₄₃, X₄₄, X₄₅, X₅₃, X₅₅, X₅₆, X₅₈, X₆₀ and X₆₁ are any amino acid; X₄₃, X₄₆, X₄₉, X₅₀ and X₅₄ are hydrophobic amino acids; X₄₇ and X₅₉ are cysteine; X₄₈ is a polar amino acid; and X₅₁ ,X₅₂ and
- 15 X_{73} X_{74} X_{75} X_{76} X_{77} X_{78} X_{79} X_{80} X_{81} wherein X_{62} , X_{65} , X_{66} X_{68} , X_{69} , X_{71} , X_{73} , X_{76} , X_{77} , X_{78} , X_{80} and X_{81} are any amino acid; X_{63} , X_{70} , and X_{74} are hydrophobic amino acids; X_{64} is a polar amino acid; X_{67} and X_{75} are aromatic amino acids; and X_{72} and X_{79} are cysteines.

 X_{57} are small amino acids; or X_{62} X_{63} X_{64} X_{65} X_{66} X_{67} X_{68} X_{69} X_{70} X_{71} X_{72}

- 214. The amino acid sequence according to claim 203 wherein at least one of the sequences comprises HX₈₂X₈₃X₈₄X₈₅X₈₆X₈₇X₈₈X₈₉X₉₀X₉₁X₉₂ herein X₈₂ is proline or alanine; X₈₃ is a small amino acid; X₈₄ is selected from the group consisting of leucine, serine and threonine; X₈₅ is a polar amino acid; X₈₆ is any amino acid; X₈₇ is an aliphatic amino acid; X₈₈, X₈₉, X₉₀ is any amino acid; and X₉₁ and X₉₂ are aliphatic amino acids or
- $X_{104}X_{105}X_{106}X_{107}X_{108}X_{109}X_{110}X_{111}X_{112}X_{113}X_{114}$ wherein at least one of the amino acids of X_{106} through X_{111} are tryptophan; wherein X_{104} and X_{114} are both small amino acids; wherein X_{105} is any amino acid; and wherein at least one of X_{104} , X_{105} , X_{106} and one of X_{112} X_{113} X_{114} are cysteine residues.

- 215. The amino acid sequence according to claim 203 wherein the two amino acid sequences are connected by a peptide or non-peptide linker.
- 216. The amino acid sequence according to claim 215 wherein the linker is a peptide consisting of about 2 to about 16 amino acids.
- 5 217. The amino acid sequence according to claim 215 wherein the linker is a non-peptide.
 - 218. The amino acid sequence according to claim 217 wherein the linker is dialdehyde.
- The amino acid sequence according to claim 203 wherein the amino acid sequence is selected from the group consisting of

DYKDDDDKFHENFYDWFVRQVSGSGSGLDALDRLMRYGEERPSLA AAGAP,

DYKDDDDKFHENFYDWFVRQVSGGSHLCVLEELFWGASLFGYCSG AAAGAPVPYPDPLEPRAA,

DYKDDDDKFHENFYDWFVRQVSGGSGGSGSHLCVLEELFWGASL FGYCSGAAAGAPVPYPDPLEPRAA,

DYKDDDDKFHENFYDWFVRQVSGGSGGSGGSGSHLCVLEELFWG ASLFGYCSGAAAGAPVPYPDPLEPRAA,

AQPAMAFHENFYDWFVRQVSGGSFHENFYDWFVRQVSAAAGAPVP 20 YPDPLEPRAA, AQPAMAFHENFYDWFVRQVSGGSFHENFYDWFVRQVSGGSFHENF YDWFVRQVSAAAGAPVPYPDPLEPRAA,

AQPAMAFHENFYDWFVRQVSGGSGGSFHENFYDWFVRQVSAAAG APVPYPDPLEPRAA,

5 AQPAMAFHENFYDWFVRQVSGGSGGSGGSFHENFYDWFVRQVSAA AGAPVPYPDPLEPRAA and

AQPAMAFHENFYDWFVRQVSGGSGGSGGSGGSFHENFYDWFVRQV SAAAGAPVPYPDPLEPRAA.

- 220. A nucleic acid sequence encoding amino acid sequence which binds to IR at Site 1 and/or Site 2, with the proviso that the sequence is not insulin, IGF, or fragments thereof.
- The nucleic acid sequence according to claim 220 wherein the nucleic acid sequence encodes for an amino acid sequence selected from the group consisting of FYDWF, FYEWF, FHENFYDWF, FHENFYDWFVRQVSK, DYKDVTFTSAVFHENFYDWFVRQVSKK,GRVDWLQRNANFYDWFV AELG and APTFYAWFNQQT.
 - 222. The nucleic acid sequence according to claim 220 wherein the nucleic acid sequence encodes for an amino acid sequence selected from the group consisting of DYKDFYDAIDQLVRGSARAGGTRDKK and KDRAFYNGLRDLVGAVYGAWDKK.
 - 223. The nucleic acid sequence according to claim 220 wherein the nucleic acid sequence encodes for an amino acid sequence selected from the group consisting of SFYEAIHQLLGV,

1 or Site 2.

NSFYEALRMLSS, SLNFYDALQLLA, SSNFYQALMLLS, SDGFYNAIELLS, HETFYSMIRSLA, HDPFYSMMKSLL and WSDFYSYFQGL.

- 224. A kit for identifying a compound which binds IGF-1 receptor, comprising a IGF-1 receptor and an amino acid sequence selected from Formulas 1-10, or the amino acid sequences of Figures 9-11, which bind to the receptor at Site
 - 225. The kit according to claim 224, wherein the amino acid sequence comprises the amino acid sequence FYDWF.
- The kit according to claim 225, wherein the amino acid sequence comprises the amino acid sequence SAKNFYDWFVKK.
 - 227. The kit according to claim 226 wherein the amino acid sequence comprises the amino acid sequence FYSLLASL.
 - 228. The kit according to claim 227 wherein the amino acid sequence comprises the amino acid sequence QMKDIFYSLLASLAAKK.
- 20 229. A kit for identifying a compound which binds IR comprising IR and an amino acid sequence selected from Formulas 1-10 or the amino acid sequences of Figures 9 and 11 which bind IR at Site 1 or Site 2.
- 230. A pharmaceutical composition comprising a amino acid sequence which binds specifically to IGF-1 receptor at Site 1 and is an IGF agonist, with the proviso that the amino acid sequence is not IGF-1, insulin, or fragments thereof, and a pharmaceutically acceptable carrier.

- 231. The composition according to claim 230, wherein the peptide comprises the amino acid sequence NFYDWFV.
- 232. The pharmaceutical composition according to claim 230, wherein the peptide comprises the amino acid sequence QMKDIFYSLLASLAA.
- 5 233. A pharmaceutical composition comprising a amino acid sequence which binds specifically to IR receptor at Site 1 and is an insulin agonist, with the proviso that the amino acid sequence is not insulin, IGF, or fragments thereof, and a pharmaceutically acceptable carrier.
- The pharmaceutical composition according to claim 233, wherein the peptide comprises the amino acid sequence FYDWF.
 - 235. The pharmaceutical composition according to claim 233, wherein the peptide comprises the amino acid sequence FYSLLASL.
- A method of treating diabetes comprising administering to an individual in need of treatment a therapeutically effective amount of an amino acid
 sequence which binds IR at Site 1 and is an insulin agonist, with the proviso that the amino acid sequence is not insulin, IGF, or fragments thereof.
 - 237. The method according to claim 236 wherein the amino acid sequence is expressed by a recombinant vector administered to the individual.
- 238. The method according to claim 236 wherein the amino acid sequence is administered to the individual as a polypeptide.

- 239. A method of treating a patient with an IGF sensitive tumor comprising administering to an individual in need of treatment a therapeutically effective amount of an amino acid sequence which is an IGF-1R antagonis, with the proviso that the amino acid sequence is not insulin, IGF, or fragments thereof.
- 240. The method according to claim 239 wherein the amino acid sequence is expressed by a recombinant vector administered to the individual.
- 241. The method according to claim 239 wherein the amino acid sequence is administered to the individual as a polypeptide.
- 10 242. A method of screening for a compound which binds to IR comprising:
 - i) immobilizing IR, or a fragment thereof, on a surface;
 - ii) incubating the IR, or fragment thereof, with a known amount of labeled amino acid sequence of Formulas 1-10, or an amino acid sequence selected from Figures 10-11, which binds IR and a compound to be screened under conditions which provide for binding of the labeled amino acid sequence to bind IR;
 - iii) measuring the amount of labeled amino acid sequence bound to IR;
 - iv) determining from the amount of bound labeled peptide whether the compound has competitively bound to IR.
- 20 243. An amino acid sequence capable of binding to Site 1 or Site 2 of IR identified by the method according to claim 242, with the proviso that the amino acid sequence is not insulin, IGF, or fragments thereof.
 - 244. The amino acid sequence according to claim 243 wherein the amino acid sequence is an IR agonist.

- 245. The amino acid sequence according to claim 243 wherein the amino sequence binds to Site 1 of IR.
- 246. The amino acid sequence according to claim 243 wherein the amino sequence binds to Site 2 of IR.
- 5 247. A method of screening for a compound which binds to IGF-1R comprising:
 - i) immobilizing IGF-1R, or a fragment thereof, on a surface;
 - ii) incubating the IGF-1R, or fragment thereof, with a known amount of labeled amino acid sequence of Formulas 1-9, or an amino acid sequence selected from Figure 10, which binds IGF-1R and a compound to be screened under conditions which provide for binding of the labeled amino acid sequence to bind to IGF-1R;
 - iii) measuring the amount of labeled amino acid sequence bound to IGF-1R;
- iv) determining from the amount of bound labeled peptide
 whether the compound has competitively bound to IGF-1R.
 - 248. An amino acid sequence capable of bind to Site 1 or Site 2 of IGF-1R identified by the method according to claim 247, with the proviso that the amino acid sequence is not insulin, IGF, or fragments thereof.
- The amino acid sequence according to claim 248 wherein the amino acid sequence is an IGF agonist.
 - 250. The amino acid sequence according to claim 248 wherein the amino sequence binds to Site 1 of IGF-1R.

- 251. The amino acid sequence according to claim 248 wherein the amino sequence binds to Site 2 of IGF-1R.
- 252. An amino acid sequence comprising the sequence $WX_{123}GYX_{124}WX_{125}X_{126}$ wherein X_{123} is proline, glycine, serine, arginine, alanine or leucine, X_{124} is any amino acid; X_{125} is a hydrophobic amino acid; and X_{126} is any amino acid.
- 253. The amino acid sequence according to claim 252 wherein X_{123} is proline and X_{125} is leucine or phenylalanine.
- 254. A recombinant peptide library comprising members wherein the majority of the members comprise an amino acid sequence of Formula 1.
 - 255. A recombinant peptide library comprising members wherein the majority of the members comprise an amino acid sequence of Formula 2.
 - 256. A recombinant peptide library comprising members wherein the majority of the members comprise an amino acid sequence of Formula 3.
- 15 257. A recombinant peptide library comprising members wherein the majority of the members comprise an amino acid sequence of Formula 4.
 - 258. A recombinant peptide library comprising members wherein the majority of the members comprise an amino acid sequence of Formula 5.
- 259. A recombinant peptide library comprising members wherein the majority of the members comprise an amino acid sequence of Formula 6.

- 260. A recombinant peptide library comprising members wherein the majority of the members comprise an amino acid sequence of Formula 7.
- 261. A recombinant peptide library comprising members wherein the majority of the members comprise an amino acid sequence of Formula 8.
- 5 262. A recombinant peptide library comprising members wherein the majority of the members comprise an amino acid sequence of Formula 9.
 - 263. A recombinant peptide library comprising members wherein the majority of the members comprise an amino acid sequence of Formula 10.

ABSTRACT OF THE DISCLOSURE

Peptide sequences capable of binding to insulin and/or insulin-like growth

factor receptors with either agonist or antagonist activity and identified from various
peptide libraries are disclosed. This invention also identifies at least two different
binding sites which are present on insulin and insulin-like growth factor receptors
which selectively bind the peptides of this invention. As agonists, certain of the
peptides of this invention may be useful for development as therapeutics to

supplement or replace endogenous peptide hormones. The antagonists may also be
developed as therapeutics.

į		Ratios ov	Ratios over Background	pun	Comparisons	isons
Clone	Sequence	E-Tag	E-Tag IGER IR		ICER/IR IR/ICER	IR/ICED
Design	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX	e ;	; ; ; ;			
R40-3-40B2-IR	IRDMHYVWVQDRDRYINGVROWYISDRYNPGSAFYRWFID	۶ 40	σ	c	u	(
DAOLALADOTO TO	TOWN THE PROPERTY OF THE PROPE	}		7		7.0
VI-21006-6-04V	KMGLQALAHIKKSAGPIFLSSGSVIKGSEGDPFYAWFRLQ	60.4	12.9	2.0	6.5	0.2
R40-4-40G11-IR	MPVSLFRRVWDYRDGEHETLESHYVVPOAALDRLFYSWFS	5,2 A	52 K 37 E	c	a at 0 c	

Figure 1A

		Ratios ov	Ratios over Background	pun	Comparisons	risons
Clone	Sequence	E-Tag	E-Tag IGFSR IR	IR	IGFR/IR IR/IGFR	IR/IGFR
Design	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX	!	!	:	!	;
R40-3-D5-IGFR	PLYGGGIHLYYPGTMGYVPGFPRQVKVLGDADKNFYDWFM	!	1	1	1	1
R40-3-A6-IGFR	YRGMLVLGRISDGAGKVASEPPARIGQKVFAVNFYDWFV	ı	1	;	1	;
R40-X-R35-IGFR	SGCCRLLGLRWMFIVIVGWSGALVCQSAASAAGFYDWFV	1	1	,	1	!

Figure 1B

		Ratios ov	Ratios over Background	pun	Comparisons	isons	
Clone	Sequence	E-Tag	IGFSR	띪	IGFR/IR	IR/IGFR	
Design	XXXXXXXXXXXXXXXXX) !	:	1 1	:	1	
R20a-3-20D3-IR	IGGQGQHQDGNFYDWFVEALA	46.3	36.2	7.0	5.2	0.2	
R20a-3-20F1-IR	VFWNCRSQQLDFYEWFEQAA	49.0	26.0	2.8	9.3	0.1	
R20a-3-20H1-IR	RVAGAISAPGLVSNKQDGLFYSWFRE	45.6	35.3	3.3	10.7	0.1	
R200-3-20D1-IR	VLQARHGCDSVSDCFYEWFA	50.8	37.5	3.0	12.5	0.1	
R20β-4-B12-IR	GAFYRWFHEALVGSERVPDV	41.9	2.9	5.7	0.5	2.0	
R20β-4-H3-IR	HEAFYDWFSALVDGGYELMG	13.9	5.8	2.4	2.4	0.4	
R20β-4-D10-2-IR	RIGGGWARSEGFYEWFVREL	21.5	7.3	2.9	2.5	0.4	
R20ß-4-C8-IR	LPAGGA?GFA?RGFYEWFES	44.9	31.1	9.6	3.2	0.3	
R20β-4-E7-IR	GHSWALVRHVDRLFYEWFDL	45.0	18.8	5.9	3.2	0.3	
R20\$-4-E7-2-IR	LGTSAGQGVGHRAFYQWFQS	45.0	18.8	5.9	3.2	0.3	
R208-4-G3-IR	RGGGTFYEWFESALRKHGAG	38.6	7.5	2.0	3.8	0.3	
R20β-4-H6-IR	NSSGQQVVGLTFYSWFASQV	14.8	7.6	2.0	3.8	0.3	
R20B-4-G11-IR	FYGWFSRQLSLTPRDDWGLP	39.4	7.5	1.9	3.9	0.3	
R20B-4-G8-IR	RMFYEWFWSQMGAGPTEGSA	41.2	15.1	3.4	4.4	0.2	
R20β-4-H9-IR	IGGQGQHQDGNFYDWFVEALA	43.1	8.8	2.0	4.4	0.2	
R20β-4-H8-IR	RDKPTDQEEQNWSFYEWFRH	47.9	43.7	9.3	4.7	0.2	
R20β-4-B8-IR	WSALLSVMDTGFYAWFDDAV	44.0	40.1	8.4	4.8	0.2	
R20β-4-E2-IR	SRDQTNFTFNSAGFYGWFER	16.3	13.9	2.4	5.8	0.2	
R20B-4-F4-IR	GVGTLTMSSDAFYTWFV	15.3	5.9	1.0	5.9	0.2	
R20β-4-A8-IR	IGGSFVEFYGWFNDQV	43.3	36.0	6.0	6.0	0.2	
R20B-4-C4-IR	DIGSDGHGRRWDSFYRWFEM	17.3	26.8	4.3	6.2	0.2	
R20β-4-D7-IR	VLQARHGCDSVSDCFYEWFA	44.8	36.2	5.6	6.5	0.2	
R20B-4-D2-IR	DPERMQSDVGFYEWFRAAVG	31.2	29.4	2.9	10.1	0.1	

Figure 1C

		Katios ove	Katios over Background	pun	Comparisons	Suosi
Clone	Sequence	E-Tag	E-Tag IGFsR IR	띰	IGFR/IR IR/IGFR	IR/IGFR
Design	XXXXXXXXXXXXXXXXX	1	1	1		t t
R20-4-B9-IGFR	DPERMQSDVGFYEWFRAAVG	40.1	40.1 16.6	:	1	ı
R20-4-F8-IGFR	DIGSDGHGRRWDSFYRWFEM	39.2	39.2 13.9	i i	i i	1
R20-4-G12-IGFR	PFYQWFLDQSVGGSRGGGLR	36.7	8.0	1	1	1
R20-4-D10-IGFR	AVAPLSVRGRDSGFYSWFSS	40.2	4.1	!	1	ŧ

Figure 1D

Clone	Sequence	Ratios ov	Ratios over Background E-Tag IGFsR H	und IR	Comparisons IGFR/IR	risons IR/IGFR
Design	XXXXXXXXXXNFYDWFVXXXX			: :	:	;
A6S-3-E12-IR	GRVDWLQRNANFYDWFVAELG	26.2	1.3	8.0	0.2	6.2
A6S-2-C1-IR	RMYFSTGAPQNFYDWFVQEWD	41.2	•	7.0	0.2	5.4
A6S-1-A7-IR	HHTQGLQVQRNFYDWFVNELR	47.2	2.3	11.1	0.2	4.8
A6S-2-C8-IR	MHRMQHDGTSNFYDWFVLQWA	44.9	1.5	•	0.3	3.7
A6S-3-E10-IR	AMHVVAQGGPNFYDWFVRELR	46.9	1.6	5.0	0.3	3.1
A6S-2-D5-IR	AIQMNGNLAFNFYDWFVRELT	31.9	1.2	3.7	0.3	3.1
A6S-1-B2-IR	TDRKSVQEPRNFYDWFVWAAR	31.6	1.8	•	0.3	2.9
A6S-1-A4-IR	PHGHRGFAQSNFYDWFVTQEE	43.3	•	9.2	0.4	2.6
A6S-4-G3-IR	RLASASVPGQNFYDWFVDQLL	31.3	2.3	5.1	0.5	2.2
A6S-4-H8-IR	RQSEFSTLNSNFYDWFVRELE		•	3.6	0.5	2.1
A6S-3-E11-IR	GQAQLSIRDVNFYDWFVQQLV	26.3	2.3	4.4	0.5	•
A6S-1-A1-IR	MSEPAVGVNGNFYDWFVAQLF	36.9	٠	6.5	9.0	1.8
A6S-2-C9-IR	VGTGRARLDRNFYDWFVGQYS	43.6	1.3	2.3	9.0	1.8
A6S-2-C4-IR	SREAVQKRNANFYDWFVQQLS	34.5	•	9.6	9.0	1.7
A6S-4-H10-IR	LAQFAGSRNQNFYDWFVEQLG	39.2	4.4	6.9	9.0	1.6
A6S-4-G7-IR	GQEYFDQMGLNFYDWFVRELD	19.1	1.4	2.2	9.0	•
A6S-4-H2-IR	RQPSQPPHGSNFYDWFVEAIN	25.5	2.6	3.9	0.7	1.5
A6S-2-C3-IR	LMQSLGSGSTNFYDWFVQQMV	31.1		•	0.7	•
A6S-2-C11-IR	DQQRSACDGTNFYDWFVCQLS	20.9	3.3	•	0.7	1.4
A6S-3-F3-IR	LDGTKACQRVNFYDWFVCQTE	37.1	3.0	4.2	0.7	•
A6S-3-E5-IR	PEARRTVVHSNFYDWFVAQLS	31.6	•	٠	•	
A6S-1-B7-IR	PWMLSVGIQDNFYDWFVGLDS	49.2	•	2.3	0.7	
1	S	37.2	5.0		0.8	1.3
A6S-4-G6-IR	TLEREGEFSGNFYDWFVEQLH	16.8	3.1	4.0	0.8	
-2-C5-	DRQSIGSVHGDFYDWFVSALG	29.7	•	•		1.3
-3-	DWDKLGSLSENFYDWFVDQLA	29.7	•	3.0		1.3
1	VRVVLNQSGRNFYDWFVIQLE	42.9				
A6S-3-E4-IR	MASWOSRTPDNFYDWFVRELS			•		
			,	•	•	T . T
	Figure 1E					

Figure 1E

		Ratios ove	Ratios over Background	pun	Comparisons	risons
Clone	Sequence	E-Tag	IGFsR	R	IGFR/IR	IR/IGFR
Design	XXXXXXXXXXNFYDWFVXXXX	I I	1 1	!	:	1
A6S-3-E9-IR	TTCHPRGEDCNFYDWFVLQLR	36.6	9.0	8.9	1.0	1.0
A6S-3-E1-IR	VRGNDSVLRANFYDWFVDQLS	36.7	6.8	6.9	1.0	1.0
A6S-4-H12-IR	TPRSQVRSDHNFYDWFVYQLA	46.3	6.1	5.8	1.1	1.0
A6S-2-D3-IR	ESLTGSRPDRNFYDWFVQQTS	37.0	5.3	5.1	1.0	1.0
A6S-3-E8-IR	PQSLTEVRTGNFYDWFVVQLH	42.7	5.2	5.1	1.0	1.0
A6S-1-A12-IR	DVGMGRVKETNFYDWFVRQLI	39.7	2.1	2.1	1.0	1.0
A6S-4-H3-IR	GADDIRSLNTNFYDWFVNQLS	18.6	3.1	2.9	1.1	6.0
A6S-3-F7-IR	GVSIQAGYKTNFYDWFVEAVR	46.2	2.3	2.1	1.1	6.0
A6S-2-D8-IR	VGEHRQMSVGNFYDWFVMQIA	31.2	2.0	1.7	1.2	6.0
A6S-3-F10-IR	GSSLGRSGPGNFYDWFVDQLE	39.0	5.9	4.5	1.3	0.8
A6S-4-G11-IR	HRQQDVVRQGNFYDWFVQALE	44.8	4.3	3.3	1.3	0.8
A6S-2-D2-IR	QDTFLTAREGNFYDWFIRALE	33.5	3.6	2.7	1.3	. o
A6S-4-G8-IR	EAIMREEGQANFYDWFVRQLE	11.1	2.5	1.9	1.3	0.8
A6S-4-H6-IR	VCDVSTGGGTNFYDWFVCQVG	22.4	2.4	1.9	1.3	0.8
A6S-2-D10-IR	PQPRSASTPLNFYDWFVQATG	41.3	2.1	1.7	1.2	0.8
A6S-3-F4-IR	GVSRGSGGDPNFYDWFVMQLR	37.0	13.5	9.9	1.4	0.7
A6S-4-G9-IR	GPGRHDSSRGNFYDWFVEQLA	36.2	11.8	7.8	1.5	0.7
A6S-3-F5-IR	ERFALEVQGSNFYDWFVRQVI	48.1	7.2	4.8	1.5	0.7
A6S-4-H1-IR	NLKSSATVGGNFYDWFVEQL	18.3	3.6	2.6	1.4	0.7
A6S-3-F6-IR	MEGPPAGGPLNFYDWFVAQVD	18.7	2.9	1.9	1.5	0.7
A6S-3-F11-IR	RLDVAGHRGGNFYDWFVKQLH	33.8	2.0	1.4	1.4	0.7
A6S-2-C6-IR	PWSDHEALNQNFYDWFVSQVL	46.7	19.2	12.1	1.6	9.0
A6S-4-G4-IR	EDRLGNGESTNFYDWFVRQLA	36.9	18.2	10.7	1.7	9.0
S	GKLVASTLDDNFYDWFVRQLS	32.8	12.8	7.9	1.6	9.0
A6S-2-D7-IR	SGPVVQTQNGNFYDWFVHQLR	33.2	12.0	7.1	1.7	9.0
A6S-4-G10-IR	VDRAGPAGSDNFYDWFVAQLD	33.9	10.8	6.8	1.6	9.0
-3-F9	SLGRNDRPDENFYDWFVSQVQ	44.3	9.6	5.7	1.7	9.0
A6S-3-F2-IR	RVMATANAPMNFYDWFVVQLQ	23.2	4.3	2.5	1.7	9.0

Figure 1E (Con't)

		Ratios ove		pun	Comparisons	isons	
Clone		E-Tag	IGFSR	ĸ	IGFR/IR IR/IGFR	IR/IGFR	
Design	XXXXXXXXXXNFYDWFVXXXX	1		!	i I	1	
A6S-4-G1-IR	NGVERAGTGDNFYDWFVAQLH	36.2	31.8	15.7	2.0	0.5	
A6S-1-A3-IR	PFACKGDKTGNFYDWFVSLTG	39.9	12.6	6.0	2.1	0.5	
A6S-3-F12-IR	GMPQEYMDQVNFYDWFVAQVD	41.4	7.4	4.0	1.9	0.5	
A6S-4-G2-IR	MGTPAVGDGANFYDWFVRQLG	26.7	7.0	3.5	2.0	0.5	
A6S-1-B1-IR	SKCKAWYGANNFYDWFVWQVD	30.6	3.7	1.9	1.9	0.5	
A6S-2-D11-IR	EAASLGSQDRNFYDWFVRQVV	48.4	37.4	13.5	2.8	0.4	
A6S-2-D1-IR	VERSASSQDGNFYDWFVVQIR	37.8	30.6	12.0	2.6	0.4	
A6S-3-E2-IR	TSEVQRRSQDNFYDWFVAQVA	33.1	24.7	9.8	2.5	0.4	

Figure 1E (Con't)

		Ratios ov	Ratios over Background	punc	Comparisons	isons
Clone	Sequence	E-Tag	IGFSR	2	IGFR/IR IR/IGFR	IR/IGFR
Design	XXXXXXXXXXNFYDWFVXXXX	:	1	1	1	1
A6S-4-E4-IGFR	ERSAAGFREGNFYDWFVAQVN	27	32	1	;	i 1
A6S-2-D2-IGFR	RAERGSMRDSNFYDWFVQQLP	36	30	1	ſ	ı :
A6S-2-F2-IGFR	LAMSVASRPANFYDWFVAQIV	35	30	1	1	!
A6S-4-F3-IGFR	HNSSSPMRTGNFYDWFVQELR	26	3.0	;	I I	1
A6S-4-G4-IGFR	SALSGPVQPINFYDWFVTGM	26	30	1	ţ	i t
A6S-4-G3-IGFR	GAQAIREIHHNFYDWFVAQVT	2.1	29	t I	1	1
A6S-2-H2-IGFR	RGORESDSGINFYDWFVGAIR	4 0	28	1	t t	1 1
A6S-2-E3-IGFR	VQEGLSGMEGNFYDWFVDQLF	36	28	1	1	1
A6S-4-C6-IGFR	RLDRSSTSGVNFYDWFVAQVG	25	28	1	1	ŧ I
A6S-4-F5-IGFR	GSQHSGREPHNFYDWFVAQVG	24	28	1	1	1
A6S-4-H3-IGFR	GRGDQRHETTNFYDWFVRELQ	20	28	i t	1	1
A6S-4-H4-IGFR	PRMVEKPSEDNFYDWFVTQLS	20	28	1	1	t I
A6S-2-H1-IGFR	RVGIQVDPHTNFYDWFVIQLT	42	27	1	i	i 1
A6S-4-E6-IGFR	RSSGGLLSQGNFYDWFVSQLE	24	26	ŧ	î t	ŧ
A6S-4-B6-IGFR	SDARQAGLQENFYDWFVSQVR	23	26	1	i	1
A6S-4-D2-IGFR	PPYRSSRLGENFYDWFVMQVR	19	26	1	1	ŧ
A6S-4-G5-IGFR	QEVTRTRDDKNFYDWFVSQIF	18	26	!	1	!
A6S-2-A3-IGFR	SRAPYGSTAGNFYDWFVQAVS	3.7	25	1	;	t 1
A6S-4-E2-IGFR	? DGQSVSSKGNFYDWFVQQMT	25	25	1	1	;
A6S-4-G6-IGFR	RLMGGIAEPQNFYDWFVREVA	20	25	1	1	: 1
A6S-4-G2-IGFR	SAGHHMPRESNFYDWFVDQVV	25	24	1	1	1
A6S-4-D6-IGFR	LGAAETWDGINFYDWFVKQVS	22	24	ŀ	t t	1
A6S-4-F4-IGFR	VGHSGVPPYPNFYDWFVMQVS	22	24	1	i	:
- 4	VTMLDKGAQDNFYDWFVREVA	21	24	1	1	t i
- 4	HHSPGNEHGYNFYDWFVLQVA	19	24	1	1	i
A6S-4-H6-IGFR	GSIAQLIMRANFYDWFVEQTN	18	24	i	1	1
A6S-4-F6-IGFR	LKGSSQPLSVNFYDWFVQQIK	1.7	24	1	1	l f
A6S-3-H1-IGFR	PASNKNSLAENFYDWFV <u>Q</u> QTR	30	23	r r	1	:

Figure 1F

Ratios over Background		Comparisons	Sons			
Clone	Sequence	E-Tag	IGFsR	R	IGFR/IR IR/IGFR	IR/IGFR
negran	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX	;	!	;	;	!
A6S-4-A6-IGFR	HVEHMAVGDGNFYDWFVVQLR	21	23	1	1	1
A6S-4-E3-IGFR	RGMTGMVGRGNFYDWFVGQLR	2.1	23	1	1	i
A6S-4-D3-IGFR	GLRSEQGNRLNFYDWFVAQIA	20	23	t I	1	!
A6S-3-E10-IGFR	RVREKLPRPENFYDWFVNQIH	23	22	1	1	1
A6S-4-D1-IGFR	SNPSRQDASVNFYDWFVREVA	22	22	1	F I	ŀ
A6S-4-B2-IGFR	QSVDLSRPDSNFYDWFVEVLS	21		i	i	!
A6S-4-A2-IGFR	IGGQGQHQDGNFYDWFVEALA	2.0	22	1	1	!
A6S-4-A5-IGFR	VEVQRHIRKDNFYDWFVKQID	19	22	1	1	!
A6S-4-C1-IGFR	CWARPCGDAANFYDWFVQQAS	16	22	!	1	1
A6S-4-B1-IGFR	RHERGKEGPGNFYDWFVSQVV	19		i	1	1 1
A6S-4-B4-IGFR	ERSPRPALASNFYDWFVQQVV	19	2.1	1	i	1
A6S-4-D4-IGFR	IARMRETFQPNFYDWFVDQLA	00	2.1	1	i	1 1
A6S-3-F8-IGFR	GRGOGLKRPDNFYDWFVAAAK	25		1	1	
A6S-3-H9-IGFR	YSIEVQDWNENFYDWFVSQLG	23		!	!	· !
A6S-3-G2-IGFR	TWMWEERKQDNFYDWFVGQLK	21		1	ı	t t
A6S-4-H2-IGFR	VTFTSAVFHENFYDWFVRQVS	19		1	!	: ;
A6S-4-A3-IGFR	LAINDLVTHKNFYDWFVDQLR	. 60		1	;	
A6S-3-G10-IGFR	GAVGLAEAGPNFYDWFVSOVO	5 t C		! !) 	l I
A6S-3-E5-IGFR	RYRGERHDGRNFYDWFVEQVN) o	l	: !	t I
A6S-3-H2-IGFR	QGAEGRLSEGNFYDWFVQAVS	2.1	0 7 6	I I i	ı I	i F
A6S-3-G3-IGFR	PRLHMGSDMGDFYDWFVVOIA		, 0	ı I	t I	! !
A6S-4-H1-IGFR	IVAGARHSEVNFYDWFVIOVR	7 7	70	;	\$ 1	l 1
A6S-4-G1-IGFR	AELVGAGVRGNFYDWFVDOLV	0 7	1 9	1 1	t I	1 1
A6S-4-A1-IGFR	DSSRIMI, GERNANDWING TO A CANADA CANA	9 !	97	!	I I	:
A6S-2-F1-TGFR	VEOVIEW CONTRACTS	17	12	1	t I	!
A6S-2-G1-TGEP	VOK VOK I VKSINF I DWF VQQAIM	30	8	1	1	: 1
A69-1-75-1750	THINDESCENAL TOWN VOVAR	30	8	1	1	1
ACC L.CJ-IGFR	⊣	2.7	7	1	1	1
A03-2-B2-1GFR	$\mathtt{EMYGDTSERVNFYDWFVSALQ}$	3.0	5	1	ţ	1

Figure 1F (Con't)

Ratios over Rackoround		Comparisons	ons			
Clone	Sequence	E-Tag	IGFsR	IR	IGFR/IR IR/IGFR	IR/IGFR
Design	XXXXXXXXXXNFYDWFVXXXX	1	1	i I	l I	1
A6S-1-D5-IGFR	RVGSGMEDLGNFYDWFVRQAQ	25	2	1	! 1	!
A6S-1-A2-IGFR	KDPVTVSQGRNFYDWFVVQIQ	20	വ	i i	1	1 t
A6S-3-E6-IGFR	DARDHGVWVMSNFYDWFVAQVS	20	S	1	1	1
A6S-1-G3-IGFR	VATVHVGGGMNFYDWFVAQVG	19	Ŋ	j T	1	!
A6S-3-G4-IGFR	CADPGACSSINFYDWFVQMRG	21	4	1	1	1
A6S-3-H8-IGFR	NPTSVQQYGVNFYDWFVNVLS	20	4	1	I I	!
A6S-3-E3-IGFR	RPSLPEVRPGNFYDWFVQSVR	19	4	I 1	i i	}
A6S-3-D9-IGFR	SLQGADFQQGNFYDWFVSELA	17	4	i i	i] 1
A6S-2-A1-IGFR	LSSRGRVTMRNFYDWFVAQVV	31	c	t t	1	:
A6S-1-H4-IGFR	HKSWTIMSPLNFYDWFVAQVE	18	n	1	1	1 1
A6S-3-C1-IGFR	RPVIGGGGTRNFYDWFVAQMI	17	m	1 1	1	1 1
A6S-3-B10-IGFR	YDQDPPYWGLNFYDWFVREVA	16	ж	i 1	i	1

		Ratios over	Ratios over Background	pun	Comparisons	isons
Clone	Sequence	E-Tag	IGFSR	¥	IGFR/IR	IR/IGFR
Parental/Design	YRGMLVLGRISDGAGKVASEPPARIGQKVFAVNFYDWFV	19.0	.4.0	:	1	;
A6L-3-D1-IR	QRGMLVRGRISHGAGKIAYEPPDCLGQKACAVNFYDWFV	22.6	19.8	26.5	0.7	1.3
A6L-4-H7-IR	<u>Q</u> RGMLLLGRISDDAGKVASEPSARRGQKVFAFNFYDWFV	37.5	3.5	4.2	0.8	1.2
A6L-4-H4-IR	YRGILVLGRISEGAGKVASEPAARIGQKVFADFYDWFV	38.5	21.1	25.8	0.8	1.2
A6L-4-E4-IR	QRGMLALGRISDGAGKVASEPPAGIGQKVFAFNFYDWFV	38.1	5.4	6.0	0.9	1.1
A6L-4-G7-IR	FRGRLVLGHFSDGAGKVGSEPAARIGQKVFDVNFYDWFV	38.6	16.2	18.5	0.9	1.1
A6L-3-C3-IR	YRGMLVLGRISDGAGKVASEPPARIGQEVFADNFYDWFV	34.7	21.8	23.1	0.9	1.1
A6L-3-B6-IR	YRGMLVLGRISDGAGEVASEPPARIGQEVFALNFYDWFV	33.1	27.8	30.3	0.9	1.1
A6L-4-G11-IR	VPWYAGSGSSSDGAGKVASEPPARIDOKVFAVNFYDWFV	27.6	2.0	2.0	1.0	1.0
A6L-4-G12-IR	YRGQLVLGRISYGAGKVGCDPPARIGQKDWAVNFYDWFV	32.0	2.3	2.3	1.0	1.0
A6L-3-A10-IR	<u>Q</u> RGLLVLGRFSDGAGNVASEPPAGIGQEVFPVNFYDWFV	21.1	2.4	2.4	1.0	1.0
A6L-4-E12-IR	<u>Q</u> RGMLVLGRISDGAGKVAAEPPDCLGQKVCAVNFYDWFV	3.1	2.4	2.4	1.0	1.0
A6L-4-E10-IR	QRGMRVLGRISDGAGKVASELPPRIGQKDFAVNFYDWFV	30.1	3.8	3.8	1.0	1.0
A6L-4-G8-IR	QRGMLVLGSISDGAGKVAYEAPARIGQTVFAVNFYDWFV	37.9	4.7	4.7	1.0	1.0
A6L-3-C12-IR	<u>Q</u> PWCAGSGRIYDGACKVASEPPAHIGQEVFAVNFYDWFV	29.5	5.7	5.7	1.0	1.0
A6L-4-H11-IR	QRGMLVLDRISDGAGKVASGPPARIGQNVLAVNFYDWFV	35.4	9.6	9.6	1.0	1.0
A6L-4-F10-IR	YRGMLVVGRISDGTGKVASQPPARIGQKVFAVNFYDWFV	31.6	10.5	10.5	1.0	1.0
A6L-4-E9-IR	YRGMLVLGRISDGAGKVASVPPAHIGQKVFAFNFYDWFV	39.8	12.9	12.9	1.0	1.0
A6L-4-H8-IR	<u>Q</u> HGMLVLGRVSVGAGKVPSEPQARIGHKVFDVNFYDWFV	38.2	14.6	14.6	1.0	1.0
A6L-3-A11-IR	YSGYAGSGSFSDGAGKVASEPPARISQEVLADNFYDWFV	29.0	17.5	17.5	1.0	1.0
A6L-4-F9-IR	YRGMLVLGRISDGAGKVASEPPARIGQKVSAVNFYDWFV	35.7	18.4	18.4	1.0	1.0
A6L-4-G2-IR	YHGKLDLGRISVGVGKVASEPPARIG <u>Q</u> KVFADNFYDWFV	29.5	21.4	20.7	1.0	1.0
A6L-4-E8-IR	YRGQAGSGVGSLTVAGKVASDPPARIGQKVFADNFYDWFV	28.7	21.6	21.6	1.0	1.0
A6L-4-H10-IR		30.0	22.1	22.1	1.0	1.0
A6L-4-G9-IR		37.1	22.6	22.6	1.0	1.0
A6L-4-F7-1R		28.6	23.6	24.4	1.0	1.0
A6L-4-E11-1R	YPWYGGSGTYLDGAGKVASEPPARIDQQVFAGNFYDWFV	38.4	26.5	26.5	1.0	1.0

Figure 1G

		Ratios ove	Ratios over Background	pun	Comparisons	risons	
Clone	Sequence	E-Tag	IGFSR	H	IGFR/IR	IR/IGFR	
Parental/Design	YRGMLVLGRISDGAGKVASEPPARIGQKVFAVNFYDWFV	19.0	.4.0	;	1	!	
A6L-4-H9-IR	YRAMLVLRRISDVAGIVDSEPPTRIGOKVFAGNFYDWFV	37.5	27.3	27.3	1.0	1.0	
A6L-4-E1-IR	YRGMLVLGRISQGAGNVASEPSSRIGQKVFAGNFYDWFI	35.4	32.6	31.4	1.0	1.0	
A6L-3-A5-IR	YRGMLVLGRISDGAGKVDYEPPARIGQKVFAGNFYDWFV	38.3	34.6	35.5	1.0	1.0	
A6L-4-G4-IR	YRGMLGLGGISAGAGIVASEPPARVGQKVFAGNFYDWFV	30.4	17.7	15.2	1.2	6.0	
A6L-4-H2-IR	YRGILFQGRIPDGAGKVASEPPTRIGERVFAVNFYDWFV	36.1	4.2	3.6	1.1	6.0	
A6L-4-E6-IR	<u>Q</u> GGMPVLGRISDGAGKVAFEPPARIGQKVFAGNFYDWFV	28.6	24.1	22.7	1.1	6.0	
A6L-4-H5-IR	YRGMLVLGRIQDGAGKVASEPPARIGQKVFTGNFYDWFV	37.2	24.6	23.1	1.1	6.0	
A6L-4-H3-IR	<u>Q</u> RGMLVLGGVSDGAGKVASDPPASIGQNVFAVNFYDWFV	37.1	9.1	7.2	1.3	8.0	
A6L-4-E5-IR	YPGMLILDRISDGASKVVSEPPASIGQKVFAVNFYDWFV	42.1	30.6	24.4	1.3	0.8	
A6L-3-C5-IR	YRGMLVLDRISDGAGKVASEQPARIGQEVYAVNFYDWFV	42.2	21.9	17.5	1.2	0.8	
A6L-4-G6-IR	YRGMLDLGRISGGVGKVASESPARIGQKVYAVNFYDWFV	29.8	4.3	2.8	1.5	0.7	
A6L-3-D4-IR	QRGMMVLGRISDGAGEVASEKVFAVNFYDWFV	39.9	12.4	8.4	1.5	0.7	
A6L-3-A7-IR	<u>Q</u> RGMLVLGRVSDGAGKVDSAPPARIGQKVFAGNFYDWFV	31.0	21.2	14.0	1.5	0.7	
A6L-3-A6-IR	QRGMLVLGRMSDGAGKVAFEPPARIGQRGFAGNFYDWFV	25.5	12.3	8.8	1.4	0.7	
A6L-4-E7-IR	QRGTLVLGRISDGAGKAASEPPARIGQNVFAVNFYDWFV	38.4	12.5	7.1	1.7	9.0	
A6L-3-C6-IR	QRGMLVLDRISDGAGKVAAEPPARIGQKVFALNFYDWFI	28.8	10.9	6.7	1.6	9.0	
A6L-4-F5-IR	<u>Q</u> RGMLVLGRISDGAGEVASEPPARIGEKVYAVNFYDWFV	33.8	6.3	4.1	1.5	9.0	
A6L-3-B7-IR	QRGILVRGRISDGAGKVGSEPPARSGEKVFAVNFYDWFI	27.6	9.4	5.0	1.9	0.5	
A6L-4-F4-IR	<u>Q</u> LGMVVLGRISDGSGKAASEPAARISQKVFAVNFYDWFV	38.9	17.6	9.4	1.9	0.5	
A6L-4-E3-IR	QRGMLVLGRISDGDGKVASEPPARIGQRVFAVNFYDWFV	38.0	6.9	3.8	1.8	0.5	
A6L-0-E6-IR	YRGMLVLGRSSDGAGKVAFERPARIGQTVFAVNFYDWFV	31.0	31.0	1.8	17.0	0.1	
A6L-0-E4-IR	YRGMLVLGRISDGAG#VASEPPARIGRKVFAVNFYDWFV	26.0	16.0	1.3	13.0	0.1	
A6L-0-H3-IR	YRGMLVLGRISGGAGKAASERPARIGQKVSAVNFYDWFV	27.0	26.0	2.0	13.0	0.1	

Figure 1G (Con't)

		Ratios o	Ratios over Background	pund	Comparisons	risons TR//CRR
Clone	Sequence vrcminispersperselgokvfavnfydwfv	E-1 ag 19	JGFSR 4	¥ ¦	IGENIA 	1101011 -
ACT-A-FR-TGFR	VRGMMVOGRISDGAGKVASVSPVRIGOKVIAVNFYDWFV	26	28	i i	1	1
A61,-2-G9-TGFR	GRI	39	22	1	1	1
AGI-4-E7-IGER	YRGMLVLGRISDGAGRVASEPQARIGQKVFAVNFYDWFV	23	22	1	1	1
A61,-4-G10-IGFR	OGGMLVPGRISDGAGKVASQPPARIGPKGFAGNFYDWFV	19	22	1	1	1 1
A6T-2-E9-IGER	YRGMRVLGRISDGAGKVASEPPTHIGQKVFPVNFYDWFV	38	21	i i	1	l t
A61,-2-D6-IGFR	YRGMLVLGRISDGAGKVGSEPAARIGQKVFALNFYDWFV	34	21	!	1	i i
A61,-3-H12-IGER	YRGOGMVLGRISDGAGKVASEPPGRIGOKVFPVNFYDWFV	24	21	l I	1	1
A61,-4-A7-IGFR		20	20	!	1	1
A6L-4-B8-IGFR	DGMLVLGRISDGAGNVASEAPARIGQKVFAVNFYDWFV	20	19	1	1 1	1
A61-4-G7-IGFR	YRGMRVRGRISDGAGKAASDPRARIGQTVLDVNFYDWFV	19	19	!	1	!
A6L-2-D9-IGFR	YRGMWVLGRISYGAGKVAYEPPARMGQKGFAVNFYDWFV	38	18	1	1	1
A6L-4-F7-IGFR	YRGMLVGGRIAGGAGIVASEPPARIGQKVFAVNFYDWFV	18	18	i	}	;
A6L-4-E12-IGFR		15	13	i	!	I I
A6L-4-H7-IGFR	YRGMLGLGRISAGAGKVASGAPARIGQEDFAVNFYDWFV	14	13	1	1	I I
A6L-4-H12-IGFR	YRGMLALGRISEGAGKVASEPPARIGQNVFAVNFYDWFV	13	12	I I	ì	1
A6L-2-A4-IGFR	YRGMLVLGRISDGAGKVASEPPARIGQKVLAVNFYDWFV	17	4	î I	1	1
A6L-3-D10-IGFR	YPGMLVPGRISDGAGEGATDPPPRIGQKVFAFNFYDWFV	16	4	1	l i	1
A6L-2-F6-IGFR	YRGMLVPGRISDGAGKVAYEPPARIGQKIFAVNFYDWFV	15	4	t t	i	I I
A6L-2-B11-IGFR	YRGVLVLGRVSDGVGKVASEPPAHRGQRVFGVNFYDWFV	26	3	t I	1	I I
A6L-1-B7-IGFR	YRRMLVLGRISDGAANVASGPPDRIGQKVFAGNFYDWFV	23	т	1	i I	1
A6L-1-D8-IGFR	YRRMLALGRFSDVTGDVASEPPAHIGQKVVAVNFYDWFV	23	Э	1	l I	1
A6L-0-A11-IGFR	YRGMVVRGRIFDGPGKVASEPRARIGQKVFAVNFYDWFV	19	ю	1	t I	î 1
A6L-3-B7-IGFR	YRGMLILGRISDGAGKVASEPPARVGQDVVAVNFYDWFV	σ	е]]	į I	I i
A6L-1-G7-IGFR	YPGRLVGGRISDGVGKVASEPPGRIGQKVFAVNFYDWFV	20	7	1	1	1
A6L-1-B9-IGFR	QRGLLVLGRIFDGAGKVASDPPARIGQKDFADNFYDWFV	18	7	1	1	1
A6L-1-C9-IGFR	YRGMLVLGRISDGAGKVAFEPPARIGONVFAVNFYDWFV	18	7	1	1	l t
A6L-0-G10-IGFR	YRCMPVLGRISDGAG#VASDRPARIGOKVFAVNFYDWFV	18	7	1	1	I I
A6L-1-G8-IGFR	YRGRLVLGRISDGAGKVAAEPPASMDSKVFAGNFYDWFV	15	7	t t	I I	j t

Figure 1H

		Ratios ove	Ratios over Background	~	Comparisons IGFR/IR IR/IO	sons IR/IGFR
Clone	Sequence	K-1 ag	IGESIA			1
Cione Design	GFREGNEYDWEVAQVT	7 0 7	1.0	12.3	0.1	12.3
E4Dα-1-B8-IR	GFREGQRWYWFVAQVT	39.6	2.0	1.5	1.3	0.8
E4Da-3-E5-IR	GFREGYFYDWFLAQVT		44.9	31.4	1.4	0.7
E4Dα-1-A1-IR	GFREGDFYEWFVAQVT	22.9	3.3	2.4	1.4	0.7
E4Dα-2-D9-IR	GFREGQFYEWFAAQVT	41.8	38.6	26.5	1.5	0.7
E4Dα-1-B3-IR	GFREGTFYDWFVAQVI	56.3	51.2	32.6	1.6	9.0
E4Dα-1-A6-IR		48.9	42.2	26.5	1.6	9.0
$E4D\alpha-1-A10-IR$		46.9	41.5	26.2	1.6	9.0
$E4D\alpha-1-A8-IR$	GFREGAFYDWFVAQVT	44.1	31.1	19.7	1.6	9.0
E4Dα-1-B1-IR	GFREGKFYQWFEAQVT	34.0	8.1	4.8	1.7	9.0
E4Dα-2-C9-IR	GFREGDFYDW FVAQV1	45.3	40.3	22.5	1.8	9.0
E4Dα-1-A3-IR	GFREGTFYEWFVAQV1	46.9	41.0	22.5	1.8	0.5
E4Dα-1-A9-IR	GFREGNFYDWFVAQV1	37.2	14.1	8.0	1.8	9.0
E4Dα-3-F3-IR	GFREGOF YEWFLAQVI	35.1	16.3	8.7	1.9	0.5
E4Dα-2-D3-IR	GFKEGZFIDWFLAXVI	33.2	5.6	2.8	2.0	0.5
E4Dα-2-D6-IR	GFKEGEFIUWFKAKVI GEDEGORVDWFRAOVT	27.8	4.5	2.3	2.0	0.5
$E4D\alpha-3-F10-1K$	GENEGAL LUMITARY.	43.8	23.8	11.4	2.1	0.5
$E4D\alpha-2-D5-1K$		25.9	7.6	3.7	2.1	0.5
$E4D\alpha - 3 - F4 - 1R$		34.6	4.0	1.9	2.1	0.5
E4Dα-3-E3-1K	GFNEGSF1GW1XAXX1	20.9	16.0	7.4	2.2	0.5
E4Dα-3-F8-IK	OF INTEREST AND A STATE OF THE	43.1	11.6	5.0	2.3	0.4
E4Dα-2-C1-1R E4Dα-1-B4-IR	GFREGIFYEWFVAQVT	45.3	6.6	2.9	2.3	0.4

Figure 11

Comparisons IGFR/IR IR/IGFR

Ratios over Background

IGFsR

IR - IR IR	9-IR GFREGDFYDWFVAQVI 6-IR GFREGDFYQWFVAQVT 9-TR GFREGGFYDWFVAQVT			2-IR GFREGKFYDWFLAQVT 12-IR GFREGKFYDWFLAQVT		10-IR GFREGRFYDWFVAQVT			37-IR GFREGHFYDWF?AQVT 38-IR GFREGEFYDWFVAQVT			311-IR GFREGIFIDWFQAQVI 39-IR GFREGNFYEWFTAQVT	X			G10-IR GFREGAFYDWFAAQVT
Clone Design E4Dα-4-H5-IR E4Dα-1-B12-IR E4Dα-4-G2-IR	E4Dα-3-F9-IR E4Dα-4-G6-IR	E4Dα-4-11 E4Dα-2-C10-IR E4Dα-1-B2-IR	E4Dα-3-F12-IR E4Dα-2-D11-IR	E4Dα-4-H2-IR E4D8-4-A12-IR	E4Dβ-4-A10-IR	E4Dβ-4-E10-IR	E4DB-4-B11-1K E4DB-4-C10-IR	E4Dβ-4-E8-IR	E4Dβ-4-G7-IR E4Dβ-4-C8-IR	E4D\$-4-A8-IR	E4Dβ-4-A9-IR	E4Dβ-4-G11-IR F4Dβ-4-B9-IR	E4DB-4-F10-IR	E4DB-4-D12-IR	E4DB-4-B8-IR	E4Dβ-4-G10-IR

3.5 2.0 2.0 1.8 1.8 1.7 1.7 1.5 1.5 1.4 1.1 1.3 1.3

> 0.7 0.7 0.7 0.7

> > 13.6

9.0 9.7 9.1 1.5

2.1

13.1

35.5 31.2 35.8 28.9 27.2

0.6

32.9

22.5

24.7

14.7

30.9

0.5

2.2 26.9 23.7 28.2

1.2

5.8 9.6 36.1

15.2 13.3 16.7

> 27.8 28.7

0.5

0.2

4.1

3.4

3.0

2.6

23.2

42.439.4

4.9

16.6 111.1 33.9 8.3 1.2

38.9 40.2 37.8 41.1

2.5 2.5 2.6 2.6

2.4

23.4 36.2 26.0 47.8

14.7

36.0 33.4 20.4 15.6

47.2

Figure 11 (Con't)

1.3

1.3 1.2 1.2 1.1 1.1 1.0 0.9
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
25.7 44.4 33.4 32.3 27.6 36.6 6.1 28.3
19.3 35.6 27.8 27.1 22.5 33.3 4.9 5.9 31.3
34.1 39.3 40.2 41.2 38.7 10.9 14.8 39.3
SAQVT DAQVT DAQVT EAQVT AAQVT AAQVT LAQVT LAQVT
GFREGSFYDWFEAQVT GFREGSFYEWFDAQVT GFREGAFYDWFEAQVT GFREGOFYDWFAAQVT GFREGDFYDWFAAQVT GFREGDFYDWFAAQVT GFREGGFYEWFEAQVT GFREGGFYEWFAAQVT GFREGGFYEWFAAQVT
GFRI GFRI GFRI GFRI GFRI GFRI GFRI GFRI
9-IR 8-IR 12-IR 12-IR 19-IR H9-IR G9-IR 12-IR '9-IR
E4D β -4-D9-IR E4D β -4-F8-IR E4D β -4-H12-IR E4D β -4-H12-IR E4D β -4-C9-IR E4D -4-H9-IR E4D -4-G9-IR E4D β -4-F12-IR E4D β -4-F12-IR E4D β -4-F12-IR

Figure 11 (Con't)

CLOIDE Sequence Design GFREGNFYDWFVAQVT E4D-2-E7-IGFR GFREGDFYDWFRAQVT E4D-2-B1-IGFR GFREGDFYDWFVAQVT E4D-2-B1-IGFR GFREGDFYDWFVAQVT E4D-2-A9-IGFR GFREGGFYDWFVAQVT E4D-2-H9-IGFR GFREGDFYDWFVAQVT E4D-2-H9-IGFR GFREGGFYDWFVAQVT E4D-2-F1GFR GFREGDFYDWFAAQVT E4D-2-F1GFR GFREGDFYDWFAAQVT E4D-2-F1-IGFR GFREGBFYDWFAAQVT E4D-2-F1-IGFR GFREGNFYDWFAAQVT E4D-2-F1-IGFR GFREGNFYDWFAAQVT E4D-2-F1-IGFR GFREGNFYDWFAAQVT E4D-2-F1-IGFR GFREGNFYDWFQAQVT E4D-2-F1-IGFR GFREGNFYDWFQAQVT	E-1 ag 20.8	IGFSK	¥ ;	GFKIK IKIGFK	Z Z Z
GFREGI GFREGI GFREGI R GFREGI GFREGI GFREGI R GFREGI R GFREGI R GFREGI R GFREGI	•		;		
и и и и и	•	!		:	;
и и и и и		22.8	1	1	!
ж жж ж	21.5	22.6	!	1	1
м жж ж	22.0	22.5	!	1 1]]
~~~ ~	20.6	22.1	1	!	1
24 Z Z	17.4	21.5	i	!	1
~~~ ~~	24.2	21.2	-	1	1
~ ~ ~	19.1	20.7	1	1	1
ж ж	24.3	20.5	1	1	1 1
œ.	21.0	20.5	1	1	
œ	25.0	20.2	t t	i	1
	22.8	20.1	I I	!	;
	21.1	19.8	ł	1	
E4D-3-F9-IGFR GFREGSFYEWFQAQVT	22.6	19.7	1	1	1
E4D-1B-E5-IGFR GFREGDFYDWFLAQVT	24.2	18.8	t I	I I	1
E4D-2-F3-IGFR GFREGHFYDWFVAQVT	23.6	18.0	i	!	i i
	22.2	18.0	1	1	;
E4D-3-G10-IGFR GFREGQFYDWFAAQVT	22.1	17.6	1	1 1	1
GFREGQFYDWFVAQVT	24.6	17.5	1	;	1
E4D-2-F7-IGFR GFREGDFYQWFAAQVT	19.0	17.5	1	1	1
E4D-3-B7-IGFR GFREGNFYDWFVAQVT	23.0	16.4	1	1	1
E4D-1B-C12-IGFR GFRDGSFYDWFVAQVT	23.0	16.1	1	1	!
GFREGHFYEWFQAQVT	21.6	16.0	1	1	:
E4D-2-E2-IGFR GFREGDFYDWFSAQVT	21.9	14.1	1	i ì	!
E4D-2-D1-IGFR GFREGHFYDWFDAQVT	24.5	13.2	i	1	1
E4D-1-D4-IGFR GFREGYFYDWFKAQVT	18.9	12.4	1	t ŧ	;
E4D-1B-A10-IGFR GFREGHFYDWFEAQVT	23.9	10.8	1 .	;	1
E4D-1B-A3-IGFR GFREGDFYDWFEAQVT	22.2	10.8	1	1	1
E4D-1-B5-IGFR GFREGTFYDWFVAQVT	19.0	10.8	1	;	;

Figure 1J

		Ratios ov	er Backgro	pun	Comparisons	risons	
Clone	Sequence	E-Tag	E-Tag IGFSR IR	¥	IGFR/IR	IR/IGFR	
Design	GFREGNFYDWFVAQVT	;	:	1	1	ţ ţ	
E4D-1B-B8-IGFR	GFREGDYYGWFEAQVT	23.8	10.7	1	1 1	1	
E4D-1-G7-IGFR	GFREGDFYAWFMAQVT	14.3	10.5	1	t r	1	
E4D-1B-A11-IGFR	GFREGNFYEWFLAQVT	24.0	10.0	1	!	i i	
E4D-1-C3-IGFR	GFREGSFYDWFDAQVT	15.8	9.3	ı	1	į	
E4D-2-H1-IGFR	GFREGNFYDQFVAQVT	19.6	4.9	ı	1	1	
E4D-1-C2-IGFR	GFREGHFYEWFAAQVT	11.5	4.5	;	i	!!	
E4D-1B-A12-IGFR	GFREGNFYEWFVAQVT	18.4	3.5	1	i i	!	
E4D-1B-A1-IGFR	GFREGKFYDWFVAQVT	22.5	2.9	1	i !	! !	
E4D-2-A3-IGFR	GFREGMFDVQLLAQVT	22.7	2.1	<i>!</i>	1	ŀ	

Figure 1J (Con't)

Control of the contro

		Ratios ove	Ratios over Background	pun	Comparisons	isons	
Clone	Sequence	E-Tag	IGFSR	K	IGFR/IR	IR/IGFR	
Design	XXXXXXFHENFYDWFVRQVSXXXXXX] 	1	1	1	1	
Parental	VTFTSAVFHENFYDWFVRQVS	29.8	17.5	16.3	1.1	6.0	
H2CA-4-F11-IR	TYKARFLHENFYDWFNRQVSQYFGRV	37.7	2.2	18.1	0.1	8.2	
H2CA-4-E10-IR	QRLSLHEQFYDWFVGQVSPLGAGG	31.2	4.4	18.8	0.2	4.3	
H2CA-4-G3-IR	GGGKVNFHEDFYGWFVQQFSGVGSDR	36.1	13.4	25.7	0.5	1.9	
H2CA-3-A11-IR	LVGDAPFHEDFYDWFARQVFGCCQEQ	35.6	12.1	22.0	0.5	1.8	
H2CA-4-F8-IR	TGAEVSFHENFYDWFDRQYSSWLDRD	36.0	21.1	33.5	9.0	1.6	
H2CA-4-G4-IR	QPHSSRLHESFYDWFDRQVPWYALDR	37.1	23.3	34.3	0.7	1.5	
H2CA-4-F4-IR	SRALAAVHEQFYDWFVRQVSGLDWGY	39.8	25.0	35.6	0.7	1.4	
H2CA-4-H10-IR	QPKDGTLHENFYDWFVRQVSSSGWVG	33.5	5.1	9.9	0.8	1.3	
H2CA-4-F1-IR	RGRLIQLHEDFYDWFLRQVSGMGGGS	36.1	19.6	25.1	0.8	1.3	
H2CA-3-D5-IR	QRGAPKSDENFYDWFVRQVLRFGEND	39.3	24.3	31.9	0.8	1.3	
H2CA-4-E11-IR	<u> AARTSLFHEDFYEWFDRQVRQEGMWG</u>	8.2	2.6	3.2	0.8	1.2	
H2CA-3-B6-IR	GTSNHSLHENFYDWFVRQLSSVQSSG	35.9	9.6	12.1	0.8	1.2	
H2CA-3-A9-IR	VSHVHLFHENFYDWFVRQLAAEGFSG	37.3	30.1	36.2	0.8	1.2	
H2CA-4-H5-IR	GRQDSGLHEHFYDWFSRQVQGEVALG	38.6	35.4	37.3	1.0	1.1	
H2CA-3-C9-IR	SNDERQFHETFYDWFVRQVSADGADR	29.3	5.1	5.6	0.0	1.1	
H2CA-3-A10-IR	LSTEQRFHEKFYDWFVHQVSTSGGGT	37.2	16.9	19.1	0.9	1.1	
H2CA-3-A3-IR	SLSREQFHENFYDWFARQVSELEGVV	29.5	28.6	32.2	0.9	1.1	
H2CA-4-G8-IR	IPGRRSLHENFYDWFVRQVSPGGGSA	32.4	29.1	31.6	0.9	1.1	
H2CA-4-G9-IR	TQKAQSLDEKFYDWFVRQVSGGGLTG	36.1	34.4	36.4	6.0	1.1	
H2CA-4-G10-IR	VSQLSDFHENFYGWFARQIAGQAEWT	34.2	35.5	37.7	0.9	1.1	
H2CA-4-H7-IR	NGTSQALHQNFYDWFAQQISGSEPGP	37.0	36.0	40.0	6.0	1.1	
H2CA-4-F9-IR	VGQSVTFHGDFYDWFDRQLSGSQEFG	37.5	36.7	39.5	6.0	1.1	
H2CA-4-F7-IR	TIDHHPLHEQFYDWFARQVSDLESLG	37.7	37.6	39.9	6.0	1.1	
H2CA-3-D10-IR	PNVGYAFHENFYDWFIRQVSIEEKAG	18.7	3.6	3.5	1.0	1.0	
H2CA-3-B1-IR	SRGSGVFHESFYNWFDRQVSEWI <u>Q</u> FG	26.5	21.4	21.5	1.0	1.0	
H2CA-3-A5-IR	QPVSGSVHERFYDWFVRQVSGSAGGG	32.9	22.9	22.4	1.0	1.0	
H2CA-4-F10-IR	<u>ASQLPPVYENFYEWFDRQVSLDAQRE</u>	26.6	27.7	28.5	1.0	1.0	

Figure 1K

i	,	Ratios ove	Ratios over Background	pun	Comparisons	isons
Clone	Sequence	gr - 7	ICFSK	<u> </u>	2525	INICER
Design	XXXXXXFHENFYDWFVRQVSXXXXXX	1		1	;	!
H2CA-3-D9-IR	VSGRGAFHENFYDWFVRQVFRDEQDT	36.6	30.6	30.9	1.0	1.0
H2CA-3-C2-IR	ARPPPTVHENFYDWFVRQVSETWRQD	38.3	30.7	31.0	1.0	1.0
H2CA-4-G1-IR	QGGDRLFHERFYDWFDRLVSSDSTGE	34.1	30.7	30.4	1.0	1.0
H2CA-4-E2-IR	QHIAAGLHENFYDWFIRQVSGVNVPA	33.9	31.0	31.8	1.0	1.0
H2CA-4-H9-IR	<u>©</u> PNDGLLHENFYDWFVRQVSNAVDGG	38.9	31.1	31.4	1.0	1.0
H2CA-3-D2-IR	PVEFTVYHDNFYDWFARQVSDGLGQF	33.0	31.1	29.8	1.0	1.0
H2CA-3-B3-IR	t FCVQASIHENFYDWFVRQVAENQVFS	35.3	31.4	30.0	1.0	1.0
H2CA-4-G11-IR	GRPEGSFHENFYDWFARQVSGDGAGT	37.9	31.9	31.0	1.0	1.0
H2CA-4-F2-IR	IVGASLCHESFYDWFACQVTNLQSQG	38.1	32.0	31.9	1.0	1.0
H2CA-3-C5-IR	IGLROMFHENFYDWFAREVSKEAGDG	36.9	32.3	31.6	1.0	1.0
H2CA-3-B2-IR	LGGAIEGHGNFYDWFVRQVSLDVGGE	36.6	32.7	32.5	1.0	1.0
H2CA-3-B11-IR	LNALQQLHENFYDWFGRQVSATPPGG	35.5	32.8	33.3	1.0	1.0
H2CA-4-G2-IR	VGNCDTFPENFYDWFACQVSELGGMN	35.9	33.0	33.4	1.0	1.0
H2CA-3-A4-IR	FSQDGNFHENFYDWFDRQLSLVGAGT	33.3	33.0	32.9	1.0	1.0
H2CA-4-H3-IR	PAGNRALHESFYDWFVRQVSEFQLGA	39.5	33.7	33.7	1.0	1.0
H2CA-4-G5-IR	DRLRARFNENFYDWFDRQVSGQGSMP	35.3	34.0	35.6	1.0	1.0
H2CA-4-E8-IR	VLGVAQFHDKFYDWFARQVSQLESAG	35.7	34.7	34.9	1.0	1.0
H2CA-4-G6-IR	GVVGGAFHEQFYDWFDRQVSAAFKGD	36.2	35.0	33.5	1.0	1.0
H2CA-3-B7-IR	DESEMRLHEQFYDWFARLVSLEGGSA	37.6	36.5	35.3	1.0	1.0
H2CA-3-B4-IR	EGGGVAIHENFYDWFDRQVSLQGWSD	39.8	36.5	35.1	1.0	1.0
H2CA-3-C7-IR	SRIVSRFHENFYDWFVRQVSGDAPVQ	40.2	36.7	35.9	1.0	1.0
H2CA-4-E5-IR	IPAGAQLHENFYDWFARQVSGEDGGA	37.3	37.0	36.3	1.0	1.0
H2CA-4-E7-IR	GSSAAGFDEQFYDWFDRQVSEAFRDG	39.7	37.6	37.6	1.0	1.0
H2CA-3-B9-IR	RLALRTFHQDFYDWFVRQVAAEDTDP	39.4	37.7	37.6	1.0	1.0
H2CA-4-F5-IR	<u>QGSFAVLHENFYDWFARQVSGVEGLA</u>	38.8	38.0	37.8	1.0	1.0
H2CA-3-B10-IR	QGNMSALHENFYDWFVRQVSEADRVD	41.9	38.9	38.0	1.0	1.0
H2CA-3-A12-IR	VAYPALLHEQFYDWFVRQVSAVAGTT	37.8	7.3	6.3	1.2	6.0
H2CA-3-A8-IR	PDTINSQHKNFYDWFVRQVSGVGTSS	36.8	22.5	19.2	1.2	6.0

Figure 1K (Con't)

		Ratios ove	Ratios over Background	pun	Comparisons	isons	
Clone	Sequence	E-Tag	IGFsR	ĭ	IGFR/IR IR/IGFR	IR/IGFR	
Design	XXXXXXFHENFYDWFVRQVSXXXXXX	;	:	;	:	;	
H2CA-3-D12-IR	SEDVDSRHENFYDWFVROVSGIGLQD	36.8	34.1	29.6	1.2	6.0	
H2CA-3-B5-TR	PAPADAFDHNFYDWFAROLSATTTIO	38.8	35.2	30.5	1.2	٥.0	
H2CA-4-E1-TR	MVORISIHENEYDWFVROISGSAVPP	29.8	12.5	11.3	1.1	6.0	
H2CA-3-D3-IR	GNVRGOFHGOFYDWFAROVSGSEGDA	33.1	29.9	27.5	1.1	6.0	
H2CA-4-E3-IR	PDAEKOFHETFYGWFVRQISEDSANS	33.3	32.3	30.2	1.1	6.0	
H2CA-4-E12-TR	FGRGVHCDENFYDWFVCOVSGALLEG	36.0	32.4	29.4	1.1	6.0	
H2CA-3-A6-TR	ETPI.TELHEOFYDWFVROVSGFPGGV	34.0	33.1	30.6	1.1	6.0	
HOCA-4-E9-TR	OHRGPHFHEDFYDWFVROVSSAVPSD	38.8	33.7	29.7	1.1	6.0	
H2CA-4-F3-1R	RODPGLFHDNFYDWFDRLVSAWDGQE	41.0	34.2	32.0	1.1	6.0	
H2CA-4-H6-TR	OAAVGVCNKDFYAWFACOVREDFAKA	37.1	34.5	30.8	1.1	6.0	
HOCA-4-HO-TR	RNWNLOFNENFYDWFDROVSALRGGG	41.8	35.3	32.8	1.1	6.0	
H2CA-3-D4-T8	RSEOYRFHENFYEWFDROVSRMGLLG	38.7	35.5	32.3	1.1	6.0	
H2CA-3-D1-TR	GAGGRDFDEDFYDWFVRQVSGQVTSG	34.5	35.5	31.3	1.1	6.0	
HOCA-3-C1-TR	SPEGNI,VHDOFYDWFVROLSSTSAGT	39.9	36.1	32.9	1.1	6.0	
HOCA 3 -D8 - IR	OGGIGDEDEDEYDWFAROVSRRDRAD	37.8	36.7	33.1	1.1	6.0	
H2CA - 4 - H4 - TR	I.SOGVGFOENFYEWPEROVSGWDGRD	38.5	37.0	33.7	1.1	6.0	
H2CA 1 III III	VEERSECHDINEYDWFFCOVSGOADGG	38.7	37.5	35.2	1.1	6.0	
H2CA 1 10 III	I.I.ASRAFHENFYDWFAROVSGTOPPG	38.6	38.0	34.7	1.1	6.0	
H2CA-4-E4-IN	VPDAOTFHESFYDWFVROASAGGPAD	40.3	38.3	36.1	1.1	6.0	
H2CA 3 CII III	ANOMGREHDNEYDWFDROVSRYERGT	41.9	38.4	35.0	1.1	6.0	
H2CA-4-E6-TR	PSRKDGLHOSFYDWFARQVQDMEGRA	39.3	38.8	35.8	1.1	6.0	
H2CA-3-D7-IR	OAVTRRFHENFYDWFAROVSEEGGWS	42.5	39.2	35.5	1.1	6.0	
HOCA-3-A7-TR	GYAVGOYOANFYDWFVROVDGMSNGG	35.3	15.2	11.6	1.3	8.0	
HOCA - 4-G10 - TR	GHORDI, HESFYDWFVROVSEAEGGG	37.6	19.4	15.1	1.3	0.8	
HOCA 3 - DA TR	DRPSSFTHENFYEWFAROVSOSGSSG	39.4	36.2	27.6	1.3	0.8	
HOCK O DO IN	ERTARTI, HEOPYDWPVROVSAMDGES	40.0	38.4	29.3	1.3	0.8	
HOCA-3-D11-TR	1.TSOLLSHEDFYDWFVROVSGVGGSG	38.1	32.9	27.2	1.2	0.8	
H2CA-3-C12-IR	PDRSDRIDDNFYDWFVRQVSQVINED	38.5	38 4	31.7	1.2	0.8	
	1						

Figure 1K (Con't)

		Ratios ov	Ratios over Background	pun	Comparisons	isons
Clone	Sequence	E-Tag	E-Tag IGFsR IR		IGFR/IR	IGFR/IR IR/IGFR
Design	XXXXXXFHENFYDWFVRQVSXXXXXX	1	1		1	!
H2CA-4-G7-IR	RAGGVGLHDNFYDWFVRQVSGGDSGP	35.9	35.9 34.7 23.7	23.7	1.5	0.7
H2CA-3-C6-IR	ADCYVQLHENFYDWFRRQVCNLQEGM	38.7	38.7 37.6 28.2	28.2		1.3 0.7
H2CA-3-B8-IR	RQGHAGFHDNFYDWFVRQVSGSTPQV	37.8	19.6	6.6	2.0	0.5

Figure 1K (Con't)

		Ratios ov	Ratios over Background	pun	=	isons
Clone	Sequence	E-Tag	IGFSR	꼼	IGFR/IR	IR/IGFR
Design	XXXXXXFHENFYDWFVRQVSXXXXX	1	1	!	1	1
Parental	VTFTSAVFHENFYDWFVRQVS	29.8	17.5	16.3	1.1	6.0
H2CA-4-G9-IGFR	GIISQSCPESFYDWFAGQVSDPWWCW	9.8	9.5	9.0	16.0	0.1
H2CA-4-H6-IGFR	VGRASGFPENFYDWFGRQLSLQSGEQ	4.9	10.5	0.7	14.6	0.1
H2CA-4-F-IGFR5	VGYQGQGDENFYDWFIRQVSGRLGVQ	5.5	9.7	0.8	12.3	0.1
H2CA-4-H8-IGFR	SACQFDCHENFYDWFARQVSGGAAYG	5.6	9.5	1.0	9.4	0.1
H2CA-4-F11-IGFR	SAAQLFFQESFYDWFLRQVAESSQPN	3.5	6.8	1.0	6.7	0.1
H2CA-4-F6-IGFR	AVRATRFDEAFYDWFVRQISDGQGNK	3.9	7.3	1.1	6.4	0.2
H2CA-4-F10-IGFR	VNQSGSIHENFYDWFERQVSHQRGVR	4.9	5.7	1.0	5.9	0.2
H2CA-1-A3-IGFR	APDPSDFQEIFYDWFVRQVSRMPGGG	7.7	3.8	0.8	5.1	0.2
H2CA-3-C8-IGFR	SSCDGAGHESFYEWFVRQVSGCRSV	15.1	5.6	1.2	4.8	0.2
H2CA-2-B9-IGFR	RAGSSDFHEDFYEWFVRQVSLSLKGK	9.3	7.0	1.7	4.2	0.2
H2CA-4-H4-IGFR	QAVQPGFHEEFYDWFVRQVSTGVGGG	3.9	4.1	1.0	4.2	0.2
H2CA-4-F7-IGFR	SSIGGGFHENFYDWFSRQLSQSPPLK	1.5	3.2	0.8	4.1	0.2
H2CA-3-D6-IGFR	QSPVGSSHEDFYDWFFRQVAQSGAHQ	8.3	0.6	2.2	4.0	0.3
H2CA-3-D8-IGFR	NYRRQVFNGNFYDWFDRQVFSLVTPG	10.9	7.2	1.8	4.0	0.3
H2CA-4-G11-IGFR	TLDGGSFEEQFYDWFVRQLSYRTNPD	10.8	9.5	2.5	3.9	0.3
H2CA-4-F1-IGFR	FYVQQWGHENFYDWFDRQVSQSGGAG	5.8	3.5	6.0	3.8	0.3
H2CA-3-D7-IGFR	LRRQAPVEENFYDWFVRQVSGDRVGG	13.3	3.0	0.8	3.7	0.3
H2CA-1-A7-IGFR	RCGRELYHSTFYDWFDRQVAGRTCPS	8.0	2.2	9.0	3.7	0.3
H2CA-2-B4-IGFR	CCLLCRFQQNFYDWFVCQGISRLRPL	3.5	4.1	1.1	3.6	0.3
H2CA-2-B3-IGFR	PPLASDLDVQFYGWFVQQVSPPGRGG	7.7	3.8	1.0	3.6	0.3
H2CA-2-B2-IGFR	GAPVDQLHEDFYDWFVRQVSQAATG	4.1	3.4	1.0	3.5	0.3
H2CA-3-D4-IGFR	RSASGSLPEQFYDWFVRQVSLSGTDK	17.6	13.8	4.1	3.4	0.3
H2CA-4-F2-IGFR	SRVTTVFHENFYDWFVRQLSDSAISG	9.3	12.8	4.2	3.0	0.3
H2CA-3-D11-IGFR	DERGGKFREDFYDWFVRQVSESRFGQ	12.2	6.9	2.3	3.0	0.3
H2CA-4-H9-IGFR	RGAVAGFHDQFYDWFDRQVSRVHKFG	8.7	5.6	1.9	3.0	0.3
H2CA-2-B11-IGFR	AICDAGFHEHFYDWFALQVSDCGRQS	11.9	4.6	1.6	3.0	0.3
H2CA-3-E8-IGFR	LGYQEPFQQNFYDWFVRQVSGAENAG	13.2	6.3	2.2	2.9	0.3

Figure 1L

		Ratios ove	Ratios over Background		Comparisons	sons
Clone	Sequence	E-Tag	IGFsR		IGFR/IR IR/IGFR	R/IGFR
Design	XXXXXXFHENFYDWFVRQVSXXXXXX	1 1	1	!	!!	!
H2CA-3-E6-IGFR	WRGHGTFHEDFYDWFVRQVSGSGSST	15.7	8.7	3.1	2.8	0.4
H2CA-4-F4-IGFR	GGRVGVLHENFYDWFDRQVSLRGADG	11.5	7.4	3.0	2.5	0.4
H2CA-3-D10-IGFR	CNLTAGFHEQFYHWFAIQVCGDAENA	9.4	6.8	5.9	2.3	0.4
H2CA-3-E1-IGFR	ERGEDMFHENFYDWFVRQISGRQGGG	12.5	6.4	2.8	2.3	0.4
H2CA-2-B6-IGFR	TNOGVGFYDSFYGWFVRQIQYGVDSG	18.0	6.2	2.7	2.3	0.4
H2CA-3-E11-IGFR	HLADGQFHEKFYDWFERQISSRCNDC	4.7	2.2	1.0	2.2	0.5
H2CA-4-H2-IGFR	QTFGKSLHENFYDWFVRQVSREEGGD	9.6	6.6	4.8	2.1	0.5
H2CA-3-C11-IGFR	FRTLAAQHDSFYDWFDRQVSGAAGER	9.3	3,3	1.6	2.1	0.5
H2CA-2-B8-IGFR	SASTHQFHENFYDWFVRQVSGAQKIL	14.6	7.9	3.9	2.0	0.5

Figure 1L (Con't)

Ratios over Background Comparisons E-Tag IGFSR IR IGFR/IR IR/IGFR	3 17.	.1 0.1	24.6 2.1 14.0 0.1 6.7	. v		•	. ~.		1 9	23.3 1.1 3.1 0.4 2.8	.3 21.	. 7	24.9	9.	.5 2.	26.0 12.7 24.7 0.5 1.9
	Clone XXXXXXXFHXXFYXWFXXXXX Design			$H2CB\alpha - 3 - D12 - IR$ VASCIIVANOSFYEWFREVMOG QARVGINVHQOFYEWFREVMOG H2CB α - 3 - H5 - IR	œ	H2CBα-3-A6-IR QFSAGAFHGDFYGWFRALYNG H2CBα-3-A6-IR GPFPFFHOFYEWFRVLNEP	H2CBα-3-B1-IR SKFDENEMS H3CBα-3-F8-IR DSVNSDLHRAFYGWFAEQWRA	H2CBQ-3-E11-IR GSVDREIHGPFYSWFERQLWG	H2CBa-3-G4-IR SANIFYLLICOTOTOTOTOTOTOTOTOTOTOTOTOTOTOTOTOTOTOT	H2CBa-3-C1-IR IMWPCTFQDFFYCWFQTEQGR	H2CBα-3-C3-IR VVGFLDIMING TOWN 3-G3-IR VVPKAGFHEAFYEWFRRQDRD	H2CBa-3-E4-IR QSFVTSVHTRFYAWFASALEM	H2CBa-3-G5-IR SKULGHINDOLLONG H2CBa-3-B11-IR GADTGAVHRRFYLWFFQLSGG		. ≃	

Figure 1M

E-Tag IGFR/IR IR/IGFR F.Tag IGFR/IGFR IGFR/IGFR F.Tag IGFR/IGFR IGFR/IGFR	14.5 18.9 18.9 19.4 20.1 19.1 19.1 22.0 4 22.0 7 22.5 6.7 6.7 7 22.5 8 21.0 9 21.2 9 21.2 9 21.2
S # O H F - F	H2CBα-3-E10-IR MRQRDGFNSSFYGWFAAALGE MRQRDGFNSSFYGWFAAALGE BEERKKVHSQFYSWFDRQLLG PSPNAPFHGGFYDWFDWVQGS H2CBα-3-A7-IR BDDSSTLNGRFYTWFHMQLLD QRGGGGFHEGFYSWFRSQSLL QRGGGGFHEGFYSWFRSQSLL QRGGGGFHEGFYSWFRSQSLL GGSSQAFHGAFYEWFSQLRG H2CBα-3-F9-IR ACBα-3-F9-IR ACBα-3-F5-IR H2CBα-3-F5-IR H2CBα-3-F5-IR CGSSQAFHGAFYEWFDQLVST H2CBα-3-F5-IR ARLLNIFDRGFYNWFQQLDE PSLSSNLHESFYRWFDQLVST PAFGLGFHQGFYDWFAHQLLD AGNSGVLHDRFYSWFERQLAG GGVSGVLHDRFYSWFERQLAG GGVSGVLHDRFYSWFTAQLAG GGUSGVLHDRFYSWFTAQLAG AGUSGVLHDRFYSWFTAQLAG GLGIASFHEGFYSWFTAQLGA

Figure 1M (Con't)

risons IR/IGFR	1.1	1.1	1.1	۲. ۲	1.1	1.1	1.1	9 1.1	9 1.1	9 1.1	9 1.1	1 1.0	1 1.0	0.1 0.	⊢ ,	_ ,	0.1 0.	0.1 0.		≓ '		0.1	
Comparisons IGFR/IR IR/IC	.1 0.9	.2 0.9	. o.	.1 0.9	۳. ۱	7.0	3.7 0.9	26.7 0.9	28.7 0.5	1.9 0.	33.2 0.9	29.0 1.	29.8 1.	20.3 1.	20.4 1.	22.0 1.	22.0 1.	23.2	25.2	25.6 1	24.9 1	26.1 1	
Ratios over Background E-Tag IGFsR IR	21.7 24	21.8 23	22.0 2 1 22.5 23	23.6 27	23.6 25	24.0 25		. 7.		28.7 3	30.8	30.5 2	31.4 2	20.7 2	20.9	21.2	22.5	23.4	24.3	24.5	24.9	25.6	
Ratios over E-Tag	, , ,	26.4	30.9		31.4	26.8	28.7			32.1	33.5	31.7	29.1	23.2	22.8	26.7	23.4	23.5	25.5	26.7	26.8	25.7	
																						O 17	
	WEXXXXXX	WFRGVIOG	WFAQQLAL	OWFSRQLSS	OWFREQLLG	SWFQAQVG1 SWFREOLLG	SWFSEQLSG	EWFETLMGD	IFHDKFYNWFEAQLKG	FNDOFYGWFRDLVDE	AWFEDQLVG	RWFDNALGS	DWFAEQVEA	<i>I</i> DWFAAQVRD	YSWFQRQLNG	YEWFNROLRG	YEWFSEAVAA	RLNVGFYQWFQDQLSG	YGWFEQQLSG	SLHDDFYEWFASQLRT	YSWFADLVGS	YGWFRKQLGE	, , , ,
	Sequence XXXXXXFHXXFYXWFXXXXXX	RVDAAALNAGFYEWFRGVIQG	GGAGKSFRDAFILME FGAROGFHARFYSWFAQQLAL	VLLPGVVHGGFYDWFSRQLSS	GALSDRYNNVFYDWFREQLLG	PDSFMSLHQRFYSWF'QAQVG1	KVI NAMENING. 1 COMESTICATION OF THE HEIGHRDVHARFYSWFSEQLSG	ARLLERFODPFYEWFETLMGD	RNSSGNFHDKFY	GSMSPVFNDQFY	SCTGRQFDGCFYAWFEDQLVG	GIAVQSLHDSFYRWFDNALGS	IGPPGSLHRGFYDWFAEQVEA	GAAGISFHRGFYDWFAAQVRD	GVDVTDFHKDFYSWFQRQLNG	WAGRAGIHGGFYEWFNRQLRG	LGQLAAFHLGFYEWFSEAVAA	VHSVSRLNVGF	LGLMAIFDRGFYGWFEQQLSG	VARGSSLHDDF	LGYIGALNTQFYSWFADLVGS	EDSRLRLHEGFYGWFRKQLGD	GKUNMAF ROOM
	Sex			œ						Ω		œ		æ								O-IR	0 - IR
	Clone	Design H2CBα-3-A9-IR	H2CBα-3-C11-IR	H2CBQ-3-B4-IR	H2CBα-3-G10-IR	H2CBa-3-D7-IR	H2CBa-3-E2-IR	H2CBa-3-B5-IR	H2CBa-3-C/-IA	H2CBa-3-69-1A	HZCBG-3-FFIE I	HZCBW-3 EX -II	uscha-3-E1-IR	112CBa-3-G12-IR	HZCBW-3-F7-IR	H2CBW-3-G8-IR	H2CBM-3-C6-IR	H2CB0-3-H9-IR	U2CBG-3-H8-IR	uscha-3-F2-IR	H2CBG-3-D5-IR	H2CBa-3-D10-IR	H2CBα-3-F10-IR

Figure 1M (Con't)

Ratios over Background Comparisons F-Tag IGFSR IR IGFR/IR IR/IGFR	26.0 25.8 1.0 1 26.9 26.2 1.0 1 27.2 27.7 1.0 1 27.7 28.2 1.0 1 27.9 28.8 1.0 1 28.1 28.8 1.0 1 28.1 28.8 1.0 1 28.1 28.9 1.0 1 5 28.4 29.1 1.0 1 5 28.7 28.9 1.0 1 6 29.0 28.1 1.0 1 6 30.2 30.2 1.0 1 7 30.2 31.5 1.0 1 3 32.5 31.5 1.0 1 3 32.5 31.5 1.0 1 3 20.2 19.1 1.1 1.1 1.0 1 3 20.2 19.1 1.1 1.1 1.1 1.1 1.1 1.1 1.1 1.1 1.	20.5 21.5 17.7 1.2 0.8 30.4 29.6 21.8 1.4 0.7
	Clone Sequence XXXXXXFHXXFYXWEXXXXXX Design H2CBα-3-D6-IR AGVMGGFHQEFYLWFERALSN H2CBα-3-H3-IR AGHVGQVYDGFYGWFRQLGA H2CBα-3-F4-IR FVQNIGFDYDFYGWFRQLGA H2CBα-3-E9-IR FVQNIGFLHRAFYQWFGSQVDA H2CBα-3-H10-IR AGGRKPFHDDFYGWFRQLAS H2CBα-3-G2-IR AGGRKPFHDDFYGWFRQLAS H2CBα-3-B2-IR AGGRKPFHDGFYGWFRQLAS H2CBα-3-B2-IR AGGRKPFHDGFYGWFRQLSG H2CBα-3-B2-IR RGRASTFHDGFYGWFRQLSG H2CBα-3-E6-IR SPARRVSHHDFYGWFAQLSG H2CBα-3-E6-IR SSDVGAFHSAFYDWFRQLSG H2CBα-3-B-IR SSDVGAFHSAFYDWFRQLSG H2CBα-3-B-IR SSDVGAFHSAFYDWFRQLSG H2CBα-3-B-IR SSDVGAFHSAFYDWFAQLSG H2CBα-3-B-IR SSDVGAFHSAFYDWFAQLSG H2CBα-3-B-IR SSDVGAFHSAFYDWFAQLSG H2CBα-3-B9-IR WRSEASFHVEFYSWFEEQLRS H2CBα-3-B9-IR VSRYGGQOGFYHWFSDLLKG	$H2CB\alpha-3-F1-IR$ RPSSGGLHYGFYHWFRVQEEM $H2CB\alpha-3-A11-IR$ SNIEEHFHMQFYRWFSDALGN $H2CB\alpha-3-A3-IR$ ANDCLGLHAGFYGWFACQLGG

Figure 1M (Con't)

isons 6.1 3.4 3.1 3.1 3.1 3.0 2.9 2.8 2.6 2.6 2.6 2.6 2.3 1.5
Comparisons IGFR/IR IR/IR IR
1.8 2.1 2.1 3.4 3.5 5.4 13.5 13.5 13.5 13.5 13.5 13.5 13.5 13.5
Backgrou 1.9 0.8 0.8 1.3 3.3 0.7 1.9 1.7 1.8 1.7 1.8 1.7 1.7 1.8 1.7 1.7 1.8
Ratios over Background E-Tag IGFSR IR
XXXX ALSG ARLSG ARRE SILDID AQLSG VQLGD OQLGD OQLGD AQVSG KAVSG AALGE DALAS QEAAG SLLSG SKLLSS
Sequence XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
Clone Design H2CBβ-3-E8-IR H2CBβ-4-F8-IR H2CBβ-3-C4-IR H2CBβ-3-D5-IR H2CBβ-4-G12-IR H2CBβ-4-F4-IR H2CBβ-4-F4-IR H2CBβ-4-F1-IR H2CBβ-4-F1-IR H2CBβ-4-F1-IR H2CBβ-4-F1-IR H2CBβ-4-F1-IR H2CBβ-4-G4-IR H2CBβ-4-G4-IR H2CBβ-4-F10-IR H2CBβ-4-F10-IR

Figure 1M (Con't)

		TARIOS OF	THE STATE OF THE PROPERTY OF T		Companisons	20113
	Sequence	E-Tag	IGFSR	ĸ	IGFR/IR I	IR/IGFR
Design	XXXXXXFHXXFYXWFXXXXXX	!!	1	t 1	1 1	i I
Parental	VTFTSAVFHENFYDWFVRQVS	29.8	17.5	16.3	1.1	0.9
H2CB-3-D2-IGFR	TASQECFDDGFYGWFRAWRCT	22.9	18.6	11.8	1.6	9.0
H2CB-3-C12-IGFR	SLDWRWSEEPFYRWFQRALAG	17.3	19.6	13.0	1.5	0.7
H2CB-3-B11-IGFR	CMSLSDCHRKFYGWFKSQGGE	24.6	17.1	11.9	1.4	0.7
H2CB-4-E2-IGFR	LALCRRSPGSFYGWFQAAVGC	22.4	21.0	16.5	1.3	0.8
.3-A5-IGFR	PRSATMSDGGFYWWFASQLGL	28.8	26.1	22.6	1.2	6.0
H2CB-4-G12-IGFR	LRRSSVFHDPFYE*ISRLVGG	23.7	23.8	19.4	1.2	0.8
H2CB-3-B2-IGFR	ARLQQQFHGGFYEWFRAQVSP	23.0	19.9	16.4	1.2	0.8
H2CB-3-D1-IGFR	AQLDNLCHEPFYSWFCAVTRE	21.5	19.5	15.7	1.2	0.8
H2CB-3-B6-IGFR	WTCDTAFHQDFYQWFCDKLGV	16.3	4.5	3.7	1.2	0.8
H2CB-4-F7-IGFR	GKEGFGLDRDFYWWFREQLGP	22.0	19.0	18.0	1.1	0.9
H2CB-4-G8-IGFR	GRAPSSFDCDFYCWFRNQVQS	20.2	18.6	16.5	1.1	6.0
H2CB-3-D4-IGFR	DVEAETQHRLFYAWFLSQLGS	21.9	18.3	16.9	1.1	6.0
H2CB-3-D5-IGFR	ISVTAVFHDGFYGWFNEQVSK	21.4	17.9	16.4	1.1	6.0
H2CB-4-E6-IGFR	NSEHGRLDVDFYGWFARVIQQ	19.6	15.8	14.8	1.1	6.0
H2CB-3-C2-IGFR	GPLGDGCQDGFYGWFMCQVST	18.8	12.2	10.8	1.1	6.0
H2CB-3-A6-IGFR	KRSAYNFHDPFYDWFRMQLSG	26.8	29.0	28.1	1.0	1.0
H2CB-4-H12-IGFR	ASEPGGYLDPFYGWFREQLRA	23.9	28.3	28.1	1.0	1.0
H2CB-3-B10-IGFR	NRGDGGVHSGFYNWFRLOLSG	27.1	27.5	27.3	1.0	1.0
H2CB-4-F11-IGFR	ASKGSSLHNDFYGWFAQQLAR	25.5	25.5	24.6	1.0	1.0
H2CB-4-G11-IGFR	ANVSMWIQVGFYDWFDAQLRQ	25.3	25.4	25.3	1.0	1.0
H2CB-4-E12-IGFR	RTSPGSLHDPFYDWFQQQLGG	27.8	24.9	24.7	1.0	1.0
H2CB-4-G10-IGFR	PGVMSSFHGGFYSWFREQLNG	25.1	24.6	24.2	1.0	1.0
H2CB-3-B9-IGFR	CLANSEDHDSFYGWFCQALGG	25.6	23.3	23.7	1.0	1.0
H2CB-3-B7-IGFR	GGSMGGMHGSFYEWFALQLRS	24.0	23.2	23.5	1.0	1.0
H2CB-4-H4-IGFR	RPOGGSIHAGFYOWFRDAVAG	23.5	23.1	23.8	1	0

Figure 1N

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		Ratios ove	Ratios over Background	pur	Comparisons	sons	
Clone	Sequence	E-Tag	IGFSR	IR	IGFR/IR IR/IGFR	R/ICFR	
Design	XXXXXXFHXXFYXWFXXXXXX	ì	1	;	1	J 1	
H2CB-4-H10-IGFR	GALSSLFDAAFYDWFNRQLEG	21.9	22.4	23.3	1.0	1.0	
H2CB-4-H5-IGFR	KVDLRGFHDGFYGWFARQLAG	22.3	22.3	21.6	1.0	1.0	
H2CB-4-G7-IGFR	CSGLQRCHDSFYSWFESVVRE	23.1	21.6	20.6	1.0	1.0	
H2CB-4-F4-IGFR	DSLGISFHEGFYDWFRRQLDM	21.3	20.9	21.3	1.0	1.0	
H2CB-3-D8-IGFR	SGVFNGTFYDWFRIQLGE	20.0	20.5	21.6	1.0	1.0	
H2CB-4-E4-IGFR	GYREMRSDLGFYQWFRDQLGL	21.6	20.5	21.2	1.0	1.0	
H2CB-4-E5-IGFR	SVFMQHDHVGFYAWFRSLMEE	22.0	19.9	20.9	1.0	1.0	
H2CB-4-E8-IGFR	FRHITEVDRSFYGWFVEQLRG	21.1	19.7	20.7	1.0	1.1	
H2CB-3-D12-IGFR	WAGGSDVDGSFYDWFQRLLAS	26.6	17.3	16.8	1.0	1.0	
H2CB-4-G9-IGFR	GLQNVSFHSGFYEWFARQVSQ	21.6	14.5	15.2	1.0	1.1	
H2CB-3-C8-IGFR	SRVSDPYHVGFYQWFEEVVRG	20.8	13.4	13.9	1.0	1.0	
H2CB-3-A12-IGFR	MGGATFFHTGFYDWFAAQLQH	28.6	27.5	29.5	6.0	1.1	
H2CB-3-B12-IGFR	RPASRPFHSGFYQWFADQLSH	27.8	25.2	27.1	6.0	1.1	
H2CB-3-A9-IGFR	GLAPGNFHEDFYRWFQEQTLG	27.7	24.3	25.7	6.0	1.1	
H2CB-3-A3-IGFR	TAAISDFNSLFYGWFEQLLSS	26.9	24.1	26.5	6.0	1.1	
H2CB-3-B4-IGFR	LDEDLPQHAGFYGWFAEALGV	25.8	23.8	25.3	6.0	1.1	
H2CB-4-E7-IGFR	ASHKSAFDDNFYRWFSMQLRD	24.6	21.6	24.0	6.0	1.1	
H2CB-4-G6-IGFR	HTGAGDLHGAFYNWFLEQLGG	22.4	21.1	23.0	6.0	1.1	
H2CB-4-E9-IGFR	RRGRDGFHGGFYDWFAAQLSD	24.3	20.7	22.0	6.0	1.1	
H2CB-4-H2-IGFR	GNFREAFHADFYSWFERQLQS	21.6	20.2	21.9	6.0	1.1	
H2CB-3-A10-IGFR	RDTLPAFHQHFYQWFEKQVSA	24.3	19.9	21.5	6.0	1.1	
H2CB-3-C4-IGFR	ERETAAFGQAFYQWFRDQIAG	23.1	19.2	22.0	6.0	1.1	
H2CB-3-B5-IGFR	WGEGGGFYDWFYDQLGWEPSH	24.2	18.8	20.7	6.0	1.1	
H2CB-4-G4-IGFR	SLVAADLHEGFYGWFRSQLGG	21.7	18.7	21.2	6.0	1.1	
H2CB-3-D9-IGFR	TSEVGDFHAEFYSWFEIQLGR	24.4	18.6	20.0	6.0	1.1	
H2CB-3-C3-IGFR	TGADGLLHARFYAWFEEQLRE	20.3	18.4	21.1	6.0	1.1	
H2CB-3-D3-IGFR	RRSDSSLHRSFYDWFSVQLLN	22.5	18.3	21.3	6.0	1.2	
H2CB-4-F2-IGFR	SESKYLLHSGFYGWFEAQLRG	18.0	16.8	18.3	6.0	1.1	

Figure 1N (Con't)

		Ratios ove	Ratios over Background	pun	Comparisons	sons
Clone	Sequence	E-Tag	IGFSR	Ħ	IGFR/IR IR/IGFR	R/IGFR
Design	XXXXXXFHXXFYXWFXXXXX	:	1 1	:	1 1	,
H2CB-4-H1-IGFR	HGVIRADHTGFYGWFSKQLSD	18.3	15.3	16.5	6.0	1.1
H2CB-4-F9-IGFR	LINA. VFRRGFYAWFEEQVSK	22.9	14.4	15.3	6.0	1.1
H2CB-4-E10-IGFR	LQRYIGFHDPFYDWFSRALSG	26.1	20.1	24.5	0.8	1.2
H2CB-4-F8-IGFR	MRTAELFHVGFYDWFDAQLMD	21.5	14.8	19.0	0.8	1.3
H2CB-3-A8-IGFR	WAPPDALHGQFYRWFQRQLDQ	20.7	14.7	18.2	0.8	1.2
H2CB-4-F1-IGFR	AVHAATFHDDFYRWFEQVVGS	22.2	14.6	18.8	0.8	1.3
H2CB-3-C6-IGFR	FDAVHGFDGGFYGWFKRELQR	15.7	7.8	10.2	0.8	1.3
H2CB-4-E11-IGFR	QAGGMEFHGAFYNWFLQQLSG	26.1	17.6	24.1	0.7	1.4
H2CB-3-D6-IGFR	GRSVSRMNAEFYQWFGHQLAA	21.6	13.0	18.8	0.7	1.5
H2CB-4-F3-IGFR	AAVNSLFHDEFYLWFQDQLDG	17.3	11.1	16.4	0.7	1.5
H2CB-3-A4-IGFR	QLGMDWFHADFYEWFLAQLPS	27.4	11.0	14.8	0.7	1.3
H2CB-3-B1-IGFR	RLAGSGIHEGFYGWFVDQLLA	20.0	11.0	15.2	0.7	1.4
H2CB-3-C5-IGFR	GREIGGVHDGFYDWFRQQSEQ	19.9	10.5	15.6	0.7	1.5
H2CB-4-F6-IGFR	VRSEQRFDSSFYQWFNDLLMS	18.6	10.1	14.6	0.7	1.4
H2CB-3-B8-IGFR	QSPYGFFHDGFYRWFLQQTGM	20.7	6.9	9.5	0.7	1.4
H2CB-3-C7-IGFR	FQCGAAFHVDFYRWFTCQEQF	16.2	1.8	2.5	0.7	1.4
H2CB-4-H7-IGFR	GAFGSEFHEQFYRWFEDALSF	21.8	14.1	22.7	9.0	1.6
H2CB-4-F5-IGFR	EHTSYQIHRQFYEWFDRALGR	12.9	4.0	7.2	9.0	1.8
H2CB-4-G1-IGFR	SGTAADLHSRFYGWFALQARE	20.4	10.3	19.7	0.5	1.9
H2CB-3-D11-IGFR	EGFGVLFHGQFYRWFQLQLDG	24.1	8.8	18.6	0.5	2.1
H2CB-3-D7-IGFR	QQSAGHPHSSFYLWFSELLGA	22.1	6.5	13.6	0.5	2.1
H2CB-3-C10-IGFR	YLQRAGFHRSFYGWFDQALRD	21.7	5.1	10.4	0.5	2.0
H2CB-4-E3-IGFR	MWLWATLHSDFYSWFEQVVSG	20.3	4.6	8.9	0.5	1.9
H2CB-3-C1-IGFR	GANALGFKDRFYEWFAAQLWD	22.3	6.7	15.7	0.4	2.3
H2CB-4-G2-IGFR	GSGLYVFHWGFYDWFEQQMGG	19.9	3.3	10.7	0.3	3.3
H2CB-3-A11-IGFR	LDKGWGFDLQFYRWFEAATRA	23.9	2.5	7.7	0.3	3.1
H2CB-4-G5-IGFR	QRSAVEFHADFYDWFLRLLTP	19.3	2.5	7.9	0.3	3.1
H2CB-4-F12-IGFR	DQRMGSFHGEFYRWFEETLLS	16.7	1.7	5.4	0.3	3.1

Figure 1N (Con't)

Sequence Ra-Fyxwe-Xm GRFYGWFQDAIDQLMPWGFDP IQGWEPFYGWFDDVVAQMFEE RYGRWGLAQQFYDWFDR RGRLGSLSTQFYNWFAE ASAYTPFYQWFADVVSEYMQQ PYRMEGTEKWNFYDWFVAQLQ SAVHFQFYKWFDNLLPVPLSA OPVNKSFYRWFQLVLGGSDDW QSPRASFYGWFDDVLRAAGVV TGFYEWFYEQLHSRMLPNPLD RRGVGGFYGWFSQQLQGMGVA SSQDRRFYRWFEQAIVGGRDG TLIQDQFYWWFSDLLSAEPGD IDQLDAFYRWFGONLGMGDP RGGGTFYEWFESALRKHGAG RGLDQDFYRWFGONLUGVEYDR MQGHRGFYGWFAVLEQDRGW

Figure 10

20E2Ba-3-D10-IR VRRDAGFYQWFADILTQLDFE 20E2Ba-4-G7-IR MQLQDEFYNWFRGIMLNDGQD	E-Tag Xn-FyxWF-Xm TMGTQGFYRWFQNVVKEHLSG 35.4		26.9 22.0		z 6. 6.	18/IGFR 1.2 1.1
MOLOUER INWERSTRUE	QLDFE 32.	2.7	27.3	29.1 30.7	6.0 6.0	1.1
GIRSSGFYOWFDRVLAGVGDG	GVGDG 33.	3,8	32.1	34.0	•	1.1
ANLNSQFYSWFASVTGEASPS	39	9	•		o. o	1
QSPRASFYGWFDDVLRAAGVV	38	2.5	31.6 30.8	35.9		1.0
	i KM	o R		30.7	1.0	1.0
œ	. "	9	30.5	30.7	1.0	1.0
150	INVRPW 38	8.9	30.6	30.7	1.0	1.0
20E2BQ-4-H10-IK	DSGGD 34	4.9	33.2	32.0	1.0	1.0
	GWCDD 34	4.1	33.7	32.2	1.0	1.0
	JESGSA. 37	7.7	32.0	32.7	1.0	T . 0
	GMDGD 34	4.9	33.9	33.4	1.0	1.0
24		18.2		•	1.0	0.1
		37.6			1.0	0.7
	4	10.4	36.0	35.6	1.0	0.1
	E	9.6	35.8	37.1	1.0	1.0
	e	33.1	4.6	4.6	1.0	1.0
ACL-4-FIL IN ELCONOMPYNWFAEOIEGSEGE	4.	14.1	40.0	38.1	1.0	1.0
200	4	13.1	40.1	39.0	1.0	1.0
	m	34.1	33.6	29.8	1.1	0.9
ZUEZBG-4-GZ-1K GYVEGIIIEN ZWEZGO-ZOO 20E2BG-4-H6-IR SHLTDPFYQWFVDQLRAGVRG		39.4	36.0	31.9	1.1	6.0

Figure 10 (Con't)

		Ratios ove	Ratios over Background	pun	Comparisons	isons
,	3	E-Tag	IGFSR	IR	IGFR/IR IR/IGFR	IR/IGFR
Clone	Sequence	1	1	l 1	1	!
Design	An-tyxwr-am An-tyxwr-am	38.7	35.1	32.3	1.1	6.0
20E2Bα-4-H5-IR	KSNUDAFIKWFSINIS ZAZAGO TARATATATATATATATATATATATATATATATATATAT	35.5	36.1	32.7	1.1	6.0
$20E2B\alpha - 4 - G3 - 1R$	DSDGAQFILWFEDQDACOROND	38.8	37.9	35.0	1.1	6.0
20E2Bα-4-H4-IR	PGLHKAF IQWFABAVICANILL	43.7	42.1	39.0	1.1	6.0
20E2Ba-3-C1-IR	SLGQGGF ILWFABQVGGADI	38.0	34.3	29.7	1.2	6.0
20E2Ba-4-E6-IR	CGQTQSFYQWFCEVMKVE3GD	43.8	21.8	18.2	1.2	0.8
H5-3-D5-IR		38.3	29.8	25.3	1.2	8.0
JBA5-3-D9-IR	KDVSMGSASINFIDMFVXXLO	22.4	6.2	1.9	3.3	0.3
20E2Bβ-4-G6-IR	SQAGSAFIAWFDQVDATVIOS	23.5	32.2	9.7	3.3	0.3
20E2Bβ-4-H10-1K ×B6-4-G8-TR	SNGISGFIEWFFFYQWFMD	1	1	i I	l 1	1
FP0-4-00						

Figure 10 (Con't)

Comparisons	IGFR/IR IR/IGFR	1 1 1	31.9 1.4 2.1 0.7 1.5
pund	IR	1	2.1
Ratios over Background	IGFSR	1	1.4
Ratios ov	E-Tag IGFSR IR IG	1	
	O do monto	Sequence	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
	i	Clone	Design R40-3-40H4-IR

		Ratios ov	Ratios over Background	pun	Comparisons	Sons
Clone	Sequence	E-Tag	IGFSR	IR	IGFR/IR IR/IGFR	R/IGFR
Design	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX	i i	1	!	!	1
R40-3-B6-IGFR	AETPAQVGWNRLWSVWPGEHWNTVDPFYHKLSELLRESGA	1	1 1	1	!	(1
R40-X-E5-IGFR	RHLTNÄELGVQSPEVLSRLFPDGDIFYRALSHLVRGMGPP	1	1	1	ŧ I	1
R40-X-B5-IGFR	RGGMDRQWLDVGARHRLERRSVQDNTDDFYGGLRILVDGF	1	1 1	t ì	i i	t I
R40-4-9-IGFR	GPPDSFDVTEKGDMAILNVRFDPHSLDFNDQTFYFLLDSL	i i	t 1	1	1	1
R40-3-G6-IGFR	GGTYFRGQVAQSNESLLRVNFLQLLEALAASPPRT	i I	1	1	1	t 1
R40-4-12-IGFR	APFDARLSAPRFQWSPRTWXOSLSYGEWSCGSFYDCLSSI	!	i	:	I I	1
R40-3-A5-IGFR	MGSSQFQDTRPSSGQAYSHSLDSDGWGTANWIFLRALEGL	; }	i t	!	1	1
R40-X-C6-IGFR	SGAAHEGNQGRERSTHLAANINDHLPGDAGIWLGYSWLS	; ;	1	: •	1	; I

		Ratios ove	Ratios over Background		Comparisons	sons
Š	Securence	E-Tag	IGFsR		IGFR/IR IR/IGFR	IR/IGFR
Clone	Schlichte Stranger St	!	!	1	t t	:
Design	AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	43.7	43.7 30.8 3.0 10.3 0.1	3.0	10.3	0.1
R20α-4-20C11-1R	DINGER LINGER OF THE CONTROL OF THE	46.3	39.9	3.1	3.1 12.9	0.1
R200-3-20E2-1R		48.6		2.4	16.6	0.1
R20α-4-20A12-IR	KLFT CGIQALGANLG I SGCV			4.3		0.1
R20β-4-C6-IR	FYSALWGLCGVIGCG)		r.	17.3	0.1
R20β-4-A6-IR	RGQSDAFYSGLWALIGLSDG)		1		

		Ratios ove	Ratios over Background	pun	Comparisons	isons
Clone	Sequence	E-Tag	IGFsR	¥	IGFR/IR IK/IGFR	KIGFK
Design	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX	1	!	1	1	1 1
D20-4-F11-TGFR	GFYELLGALVGERVRGTGNS	39.0	19.2	1	t 1	i i
D20-4-C7-IGFR	FRTDPFYKALLSLLGGDGSG	33.4	17.5	1	1	1 1
D20-3-F2-TGFR	DVOGNGSSGFYDGIFGLAWG	31.8	14.3	i I	1	i i
N20 3 12 1311 D20 4 - N11 - TGFP	PRYVWIRDLIGPELPHTRGD	37.8	13.5	1	1	1
DOOLALBIOLIGER	VIAVAGGPI,DPFYEGLHRLIS	37.2	10.0	i i	ì	1
N20-4-B10-IGFR	GFYRLLNELVREGGALKVGA	37.0	9.5	1	1	1
3471 - 943 - 971 -	CORGEVELLSELLGHEGGVF	34.2	9.4	1	1	i I
NZO-4-E/-1011 D20-3-H4-1GFR	DWVSGPFYRGIELLSGFOIE	30.3	7.8	!	I I	1 1
NZO-3-114 TOIN	GGSLFYEGIJRLYLGDSVVG	20.8	6.9	! 1	1	1 1
NZO 3 SZ ISIN	LINHEYAMLSDLSGVRNIFPG	32.8	6.5	1	1 1]
R20-4-E7-IGFR	LSGFYEGLFRLARRDGSGWG	35.4	6.4	1	!!	l 1
R20-4-G9-IGFR	FYDVLSALVGVELGEQGDAS	25.0	6.4	1	1	1 .
R20-4-D9-IGFR	GAGSFGREGGFYEALMQLAG	23.4	6.3	1	1	ŀ
R20-4-D11-IGFR	DDEFYSQILKLVDGSRGGRSGTQN	31.3	4.0	t 1	1	i i
R20-4-G10-IGFR	PFYMLLSRLVGGVEQEGGL	13.6	3.3	1	t I	1
R20-4-C8-IGFR	FYDAIDQLVRGSARAGGTRD	16.8	3.2	l l	i i	1

		Ratios ove	Ratios over Background		Comparisons	SOUS
Clono	Seallence	E-Tag	IGFSR		IGFR/IR IR/IGFR	R/IGFR
Clone	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX	;	1	!	1	1 1
Design	AAAACAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	33.1	32.3	1.2	27.0	<0.1
20C-3-G3-1GFR	IFISCHASHLIGIFKING HENGT	35.3	28.0	1.3	21.8	<0.1
20C-4-C/-1GFR		33.5	26.1	1.9	14.1	0.1
20C-3-F6-IGFR	DEDFORFIER LAD GOZVOGONFO	26.5	20.8	1.5	13.7	0.1
20C-3-A1-IGFR		17.7	8.8	1.2	7.6	0.1
20C-3-A4-IGFR	OFYGCLLDLSLGVFSFGWRRRCITA	37 7	7 7	.3	6.0	0.2
20C-3-E4-IGFR	FFYRCLSRLGGQLGSRLGLSC1GD		, , ,	י ע	8 6	0.4
20C-4-D11-IGFR	DLFYCMMMQLATAGVGGSLGGPVCG	6.66	7.11		, ,	
200-4-E7-TGEP	CDEVCALSELSGOPRDRMPNYPGTS	31.2	28.9	7 / 0	۲.,	0
20C-4-F/=1GFR 20C-3-B2-IGFR	GSACDGFYACLHALVQGPGEW	37.7	35.2	30.9	1.1	o. o
	ı					

		Ratios ov	Ratios over Background	pun	Comparisons	isons
Clone	Sequence	E-Tag	IGFSR	IR	IGFR/IR	IR/IGFR
Darental/Degion	AETPAOVGWNRLWSVWPGEHWNTVDPFYHKLSELLRESGA	1	!	1	1	;
	ANT.R. VGNRI, * SVWPGEOWNTVDP? YOKLYELVRESGA	40.5	6.1	40.8	0.2	6.7
DOD-4-CO-110		19.6	4.0	23.5	0.2	5.9
Bon-4-B/-IR	AFTDAHVC*TVGGI,FGRVNTWNTVDPFYAKLSELLRESGA	20.6	2.8	2.7	6.0	1.1
B6L-3-HI-IK	GONGGSAWDGTSLWSVWPGDVWNPVDPFYHKLSELLRESGA	15.5	4.1	3.0	1.1	6.0
B6L-4-E12-IN B61-4-D8-TR	AEA PAOVGWNI, OPGEHWI TVDPFYNKLSELLRESGA	36.0	9.4	8.5	1.1	6.0
77 - 73 - 7 - 170	AETPAOVGWNGLWSVWPGEH*NTVDPFYHKLSELLRESDG	37.8	24.6	20.6	1.2	6.0
B61-4-F7-11.	AETPAOVGONGLWSVWPGEHWNTVDPFYOKLFELLRESGA	5.5	2.0	1.6	1.3	0.8
B61-4-B12-TR	AETPAOVGONRLWSVWPGELWNTVDPFYHKLSELLRESGA	6.8	2.0	1.6	1.3	0.8
BGH-4-B12 IX	T*OGETPAOVSLWPGEHWNTVDPFYHRLSELLRESGA	36.4	18.7	14.2	1.3	0.8
BCI - 4 - F8 - 1R		35.6	11.4	8.6	1.3	0.8
71	VOTTPAOVGWNRLWSVGPGEHWYTDDPFYH*LSELLRESGA	7.6	2.5	1.8	1.4	0.7
AL -30 - F- 138	AETSAOVGWORLWSVWPGDHWSTLDPFYHKLSELLRESGA	11.5	2.0	1.4	1.4	0.7
B614-E10-TR	*NSPRVGWNGLWSVWPGEHWNTWDPFYNKLSELLRESGV	14.8	3.2	2.2	1.5	0.7
B614-F10-TR	AETPAOIGWNRLOSVWPGEYWNTVDPFYLKLSELLRESSP	26.2	11.5	7.2	1.6	9.0
B61,-3-F3-TR		36.0	17.1	10.1	1.7	9.0
BK14-A7-TB	AGTPAOVG*NRLWSVWPGEHWNTVDPFYNKLSELLRESGA	11.6	3.4	1.9	1.8	9.0
B614-G8-TR	D*OAWSVWPGOHWNTIDPFYHKLSELLRESGA	30.4	11.2	5.9	2.0	0.5
B61,-4-F8-IR	AETLARVGWNRMQSVWPGEHWNTVDPFYHKLSELLRESGA	35.6	12.8	7.2	2.0	0.5
B61,-4-G7-1R	AATRPOVGWNRVWSVOPGEHWNTVDPFYHKLSELLRESGS	33.5	12.9	6.4	2.3	0.4
R613-F4-TR	LTTPAOVGWNRLOSVWPGEHWNSVDPFYHKLSELLGESGA	16.9	6.3	2.7	2.4	0.4
B61,-3-H4-IB	ADNPAOVGWNRLWSVWPVEH*NTVDPFYHKLSELLRESGA	20.6	4.9	2.0	2.5	0.4
BGI3-AG-IR	AETPAOVGWNRLOSDWPGGHWNTLDPFYHKLSELLRESGA	22.4	6.3	3.2	3.2	0.3
B61,-4-D7-IR	AETSVOVGWIRLOSVWPGEHWNTVDPFYHKLSELLRGSGA	14.3	4.8	1.4	3.4	0.3
B6L-3-E2-IR	G*NSAHVGWNRLWSFWPGEHWNTVDPFYEKLSELLRVSGG	29.5	16.7	3.8	6.3	0.2

					•	
		Ratios ove	Ratios over Background	pur		ONS
	Cocnence	E-Tag	IGFsR	¥	IGFKJIK IR	IK/ICFK
Clone	SCHULLES ON CHANGE WEST WITH THE SELL RESGA	1	1	;	:	! 1
Design	AELFACTORI WESTWOOTEHWITVDPFYHKLS?LLRESGA	7.3	22.1	!	1	:
B6L-4-G6-1GFR	AEIFACAGGILLISUMPGESWNTVDPFYLKLSELL?ESGA	5.5	21.8	; 1	1	•
B6L-4-G10-1GFK	AE: FACTORING SWINNERS WILLIAM STATE AND A STATE OF A S	5.8	18.1	;	1	! !
B6L-4-G3-1GFK	A ET 1. PAYONING TO THE STATE OF THE SELL RESGA	6.7	17.4	i	i i	! 1
B6L-3-F10-1GFK	A CATE A CALCAN SAMP CEHWAT VDPFYHKLSELLRESGA	6.5	15.9	1	1	!
B6L-4-U2-1GFR	AETING OCCUMENT OF THE SELL RESGA	5.9	15.2	1	\$ •	;
B6L-3-H10-1GFK	AELERO ONGWINELWSDWPGEOWNTI, DPFYHKLSELLRESGA	6.3	14.8	1	!	:
B6L-4-B12-1GFK	CETSACVOMINET SECTION OF THE SELLRESGA	5.2	14.8	1	1	;
B6L-3-A9-1GFR	A CHARLOS WILLIAM STANDER WILLIAM STANDER SELL RESGA	5.4	11.9	!	;	:
B6L-4-C4-1GFK	AEIAAÇVOIMILEÇOTTE CETTURE A VALABLERESGA	4.4	11.1	! :	1	:
B6L-4-E3-1GFK	AEAFDQVOXILINGVIII CEIIII CEIIII CEIIII CEIII CEIII CEIII	3.0	10.4	!	!	ı •
B6L-4-A12-1GFK	A CHERT AND A CHERT CHEMINATIVE TO A CHERT A C	4.7	10.3	1	1 t	1
B6L-4-D3-1GFR	AFILE ACCUMENT WISH WAS A STREET OF THE SELLINGSDA	10.9	7.6	1	:	!
Bon-3-Alu-lera	AFTERACUCIONE WSVWPGEH*NTVDPFYHKLSELLRESGA	2.9	9.1	1	î	!
B6L-3-B9-1GFA	AETTACOVGWNSI,OSVWPGEHWNT?DPFYHKLSELLRESGA	4.5	8.8	1	1 (:
BOLL-3-A3-IGEN	A ESPA OVESNRI OSVWSGEHWNTVDPFYHKLSELLRESGA	2.4	8.0	í i	I E	i I
B6L-4-A3-1GFR	AFTPAOVGOYRLSSVWPGEHGNTVDPFYHKLSELLRESGA	4.2	7.5	1	:	:
Bon-3-Mil-1GFR	AFTPAOVGWNRLWSVWPGEHWNTIDPFY*KLSELLRESGA	2.5	7.4	1	! !	:
BOLL J-GIO ICER	DETPAHVGWNRPOSAWPGERWNTVDPFYHKLSELLRESGA	2.4	6.8	:	1	:
D61 - 4-E11-1GFP	AGTPACVGWNRLRSVOPDEHWNTVDPFYHKLSELLRESGA	2.1	6.4	i t	!	;
DOI - 4 - 1 1 2 - 1 CER	A F T D A O V GWORL W S V W P G E H W N P ? D P F Y R K L S E L L R E S G A	2.7	5.9	1	1	ı 1
B614-F12-TGFR	AETPACYGWNRLOSVWPGEHWNTVDPFYHKLSELLRESGA	1.9	3.9	1	t I	1
BEL-4-F10-TGFR	AETPACYGWNRLWSVOPGEHWNTVDPFYHKLSELLRESGA	1.8	3.6	1	t 1	<u>:</u>
B6L-3-G9-IGFR	AETPAQVGWNRLWSVWPGEHWNTVDPFYHKLSELLRESGA	1.2	2.5	1	!	1

		Ratios ove	Ratios over Background	pur	Comparisons	risons 10/10FD
Clone	Sequence	E-Tag	Grsk	¥ ;	ICFRIR	1.5101
Design	OOUUUUUUUJJJJDPFYHKLSELXXOO					6
B6Ha-3-F5-IR	GGAVAAAVVGSRADPFYHKLSELVQGS	42.7	9.6	17.9	0.1	T
DCUA-2-D10-TR	SGGGOORKAIATSDDPFYHKLSELLLGG	20.8	1.7	1.1	1.5	9.0
BORU-Z-DIO-IN	CSMADAVARAGDDDDPFYHKLSELCOGS	22.5	2.4	1.3	1.8	0.5
B6Hu-3-Filh	COLUMNICATION OF THE CASE OF T	18.2	2.3	1.2	1.9	0.5
B6HG-3-E6-IK	CONTRACTOR TO THE SELECT OF TH	44.6	5.2	2.1	2.5	0.4
B6HQ-1-B8-IA	SEVINAMATERGSPDPFYHKLSELVOGR	39.6	2.3	0.9	2.6	0.4
BORU-2-DJ-IN	CITTAENGAGDPFYHKISELGGCS	33.1	3.2	1.1	2.9	0.3
BORG-1-B3-1K	OCCUPATION OF THE SELFCIC	28.8	2.9	1.0	2.9	0.3
Bond-3-E3-In	CCEVI DA FORRDIDEVHKI, SELCOGG	17.4	6.4	2.1	3.0	0.3
BOHU-4-D9-IN	CCEVILLING CONTRACTOR DEPORTS CO	19.3	3.0	1.0	3.0	0.3
BOHU-Z-DO-IN	WI. CHARACRE PERDENDEY HKI.SELIDGG	43.1	8.7	2.8	3.1	0.3
MI - FA - C - MIDA	SRVAATKEKRPSDDPFYHKLSELLOGS	41.5	3.1	1.0	3.1	0.3
Bond-3-F7-IN	SPAKVEAEMPDSGDDFYHKLSELLASG	37.4	2.6	0.8	3.3	0.3
BONG-1-A3-IN BONG-1-H10-IP	GGAAKKTVVGSPDDFYHKLSELLOGS	50.5	29.5	8.6	3.4	0.3
DCUM-3-EE-ID	CGVGEOMEVTODGDDFFYHKLSELLWSC	48.9	19.7	5.7	3.5	0.3
Bolla-3-F3-TR	SGEOTATIEGESNDFFYHKLSELIWGS	18.1	15.6	4.3	3.6	0.3
B6Ha-4-G8-IR	GGTKAVAKVGTRDDPFYHKLSELLQGS	32.3	6.1	1.7	3.6	0.3
B6Ha-2-D1-IR	GCEVIVEEGDSADPFYHKLSELCQGS	11.7	5.4	1.3	4.2	0.2
B6Hq-3-E7-IR	GCAVVEEAERSRGDPFYHKLSELIQGC	47.0	5.6	1.3	4.3	0.2
B6Ha-2-D6-IR	GRIMAVMAAGGPDDPFYHKLSELVQGG	33.5	4.4	1.0	4.4	0.2
B6Ha-3-F10-IR	GCVVEWOKWHGASDPFYHKLSELGGCS	47.2	8.8	1.9	4.6	0.2
B6Ha-3-E8-IR	RGKTAAVIVGRPADPFYHKLSELLQGG	47.6	5.3	1.1	4.8	0.2
B6Ha-2-C10-IR	SGAKVIVVTGDSGDPFYHKLSELLQGS	46.9	5.8	1.1	5.3	0.2
B6Hα-2-C7-IR	RGIVAMVEATEVGSDHDPFYHKLSELVQGS	45.1	6.7	1.0	6.7	0.1

FIGURE 2H

		Ratios ove	Ratios over Background	nd :	Comparisons	isons
Clone	Sequence	E-Tag	IGFSK	¥	CFRIR	INIGER -
Design	OOUUUUUUJJJJDPFYHKLSELXXOO	2	t I	:	ì	
BEHG-1-A6-TR	GCKMEETETGTSDDPFYHKLSELCSGG	49.7	34.5	4.0	8.6	0.1
BEHA-2-CE-IR	RGEVATMEVPAGGDPFYHKLSELLWGS	42.6	34.2	3.3	10.4	0.1
Bond-2-CJ IN	ROTEW A FMGAGDDFYHKLSELVCG	20.7	6.6	0.9	11.0	0.1
Bond-2-C4-IN	PCMVETTAVGSGDDPFYHKLSELCOGG	47.4	32.6	2.8	11.6	0.1
Bond-2-C3-IN	WWOKKSGDGASASDPFYHKLSELIWGS	36.3	28.1	2.4	11.7	0.1
Benu-4-no in BEHu-3-F11-IR	RGMKEEVLVGGSTDPFYHKLSELLQGS	49.5	18.7	1.6	11.7	0.1
BOILE 3 FFG-TR	RCEEKOAEVGPSSDPFYHKMSELLGCR	44.6	24.2	1.7	14.2	0.1
BGHG-1-A2-TR	RGCNDDGGKGWSDDPFYHKLSELICGG	22.3	14.6	1.0	14.6	0.1
B6Ha-1-B5-IR	CCTTEMVVMDARDDPFYHKLSELVTGG	41.5	20.5	1.0	20.5	0.0
B6H8-3-G4-IR	GCKKVEAKKGNDADPFYHKLSELLQGC	36.4	28.4	36.0	0.8	1.3
B6H8-3-A10-IR	RSMMAKAIVGGPGDPFYHKLYELQFGSR	36.7	27.9	34.7	0.8	1.2
B6H8-3-D9-IR	CGGAVPDGDDPFYHKLSELMQGC	34.9	32.1	35.6	0.9	1.1
BGHB-3-A3-IR	GCEEVEAETTGHRDPFYHKLSELLQGC	36.3	33.7	37.3	0.9	1.1
B6HB-3-G1-IR	GCAEIEIAAGGGGDPFYHKLSELLQGC	34.7	33.7	35.9	0.9	1.1
B6H8-3-B3-IR	GCAEVKAVKGAGDDPFYHKLSELLQGC	35.9	35.1	37.4	0.9	1.1
B6HB-3-G11-IR	GCAAVETTINGRNDPFYHKLSELLQGCR	37.6	36.2	39.0	0.9	1.1
B6HB-3-F5-IR	CGEVTGRAGDPFYHKLSELLQGC	39.2	37.2	41.0	0.9	1.1
B6H8-3-A1-IR	GCAMVEATEGRRHDPFYHKLSELIQGC	41.3	38.0	43.0	0.9	1.1
B6H8-3-H3-IR	GCTEVVGSGDDPFYHKLSELLQGC	39.0	38.3	40.7	0.9	1.1
B6H8-3-D3-IR	GOCAMEEIIRGANDPFYHKLSELCEGG	38.8	38.4	41.3	0.9	1.1
B6HB-3-C9-IR	GCAEIVIEEGDDSDFYHKLSELLQGC	36.7	39.2	41.5	0.9	1.1
B6H8-3-F1-IR	PQCSSIKAEGGSDDPFYHKLSELLVGC	41.5	40.0	42.2	0.9	1.1
В6Нβ-3-C6-IR	GCAAVVAEASGDDPFYHKLSELLQGC	39.9	40.3	42.7	6.0	1.1

Sequency Sequency OOUUUUUUUUJJJDPFYHKLSELXOO 39.5 27.1 26.3 RGDGDPFYHKLSELZQGS 34.3 34.4 35.4 RGGDGDPFYHKLSELLQGS 35.3 35.0 35.6 SEKVVKATVGTPHDPFYHKLSELLQGS 36.8 35.4 36.5 GCAAIANATGNNDPFYHKLSELLQGS 37.7 36.4 37.6 GCAAIANATGNDPPFYHKLSELLQGS 37.4 36.5 37.2 GCAAIANGSPDPFYHKLSELLQGS 37.4 36.5 39.1 SCAAEKEVAGTARDPFYHKLSELLQGS 37.4 36.5 39.1 MCDRDGRDEQPMDFYHKLSELLQGS 37.6 39.1 39.4 MLCDRDGRDEQPMDFYHKLSELLQGS 37.8 39.0 39.4 GSVAAAKKTGSSDDFYHKLSELLQGS 37.6 39.0 39.4 GCKAVTWTWTMRSPADPFYHKLSELLQGS 37.6 39.0 39.4 GCKAVVENCHORDPFYHKLSELLQGS 39.0 39.4 41.3 GCKAVVENCHORDPFYHKLSELLQGS 39.0 40.1 40.7 GCKAVVENCHORDPFYHKLSELLQGS 40.1 40.1 41.6 GCKAVVENCHORDPFYHKLSELLQGS <th></th> <th>,</th> <th>Ratios ove</th> <th>Ratios over Background</th> <th>nnd IR</th> <th>Comparisons IGFR/IR IR/IC</th> <th>risons IR/IGFR</th> <th></th>		,	Ratios ove	Ratios over Background	nnd IR	Comparisons IGFR/IR IR/IC	risons IR/IGFR	
ROUGHNAIN OF THE PRINCES CONTRINGES CONTRINGES CONTRIBED CONTRIBECTORS	ne	Sequence	8 * 1 -7	1	: ;	1	:	
CSAVUKRATUGEDPEYHKLSELLQGS RGGGGDPFYHKLSELLQGS RGGGGDPFYHKLSELLQGS RGGAAVVKATVGTPHDPFYHKLSELLQGS GCAAIAVATGNDNDPFYHKLSELLQGCR GCAAIAVATGNDNDPFYHKLSELLQGCR GCAAIAVATGNDNDPFYHKLSELLQGCR GCAAVKETHDPPDPFYHKLSELLQGCR SCAAEKEVAGTARDPFYHKLSELLQGCR WICDRDGRDEPWHPFYHKLSELLQGCR MICDRDGRDEPWHPFYHKLSELLQGCR MICDRDGRDEPWHPFYHKLSELLQGCR MICDRDGRDEPWHPFYHKLSELLQGCR MICDRDGRDEPWHPFYHKLSELLQGCR MICDRDGRDEPWHPFYHKLSELLQGCR GCAVUTWTMRSPADPFYHKLSELLQGCR GCAVUTWTMSSDDPFYHKLSELLQGCR A1.1 41.4 41.9 11. CCDEKQRVTGGTNDDFYHKLSELLQGCR GGAAVVVAMGGNDDPFYHKLSELLQGCR GGAAVVVAMGGNDDPFYHKLSELLQGCR GCGAVVANAGNGDDPFYHKLSELLQGCR GCGTLAACAACAACAACAACACAACAACAACAACAACAACAACA	sign on the contra	DOVEMAN TANGTENDE PYHKT, SELS SGS	ο.	27.1	26.3	1.0	1.0	
RGGDGDPFYHKLSELLQGR	Hp-3-D/-1R	CONVEMBERGING TO THE SELECTION OF THE SE			35.4	1.0	1.0	
### CKRQTHDPPFYHKLSELLQGS SSKVWATVGTPHDPPFYHKLSELLQGS SSKVWATVGTPHDPPFYHKLSELLQGS SCAAIAVATGNDNDPFYHKLSELLQGCR GCAAIAVATGNDNDPFYHKLSELLQGCR GCCAAIAVETHDPPDPFYHKLSELLQGCR GCCAAIAVETHDPPDPFYHKLSELLQGCR SCAAEKEVAGTARDPFYHKLSELLQGCR #### MQRNKQQIIGTPDDPFYHKLSELLQGCR ### MQRNKQQIIGTPDDPFYHKLSELLQGCR ### MQRNKQQIIGTPDDPFYHKLSELLQGCR ### MQRNKQQIIGTPDDPFYHKLSELLQGCR ### MQRNKQQIIGTPDDPFYHKLSELLQGCR ### MCCDRGRDEQPMDPFYHKLSELLQGCR ### MCCDRGRDEQPMDPFYHKLSELLQGCR ### MCCDRGRDEQPMDPFYHKLSELLQGCR ### MCCDRGRDEPFYHKLSELLQGCR ### MCCDRGRDEPFYHKLSELLQGCR ### MCCRAVVEVKDHGDDPFYHKLSELLQGCR ### MCCNGCKAVVEVKDHGDDPFYHKLSELLQGCR ### MCCNGCKAVVEVKDHGDDPFYHKLSELLQGCR ### MCCNGCKAVVEVKDHGDDPFYHKLSELLQGCR ### MCCNGCKAVVEVKDHGDDPFYHKLSELLQGCR ### MCCNGCKAVVEVKDHGDDPFYHKLSELLQGCR ### MCCNGCKAVVEVKDHGDPFYHKLSELLQGCR ### MCCNGCKAVVEVKDHGDPFYHKLSELLQGCR ### MCCNGCKAVVEVKDHGDPFYHKLSELLQGCR ### MCCNGCKAVVEVKDHGDPFYHKLSELLQGCR ### MCCNGCKAVVEVKDHGDDPFYHKLSELLQGCR ### MCCNGCKAVVEVKDHGDPFYHKLSELLQGCR ### MCCNGCKAVVEVKDHGDPFYHKLSELLQGCR ### MCCNGCKAVVEVKDHGDPFYHKLSELLQGCR ### MCCNGCKAVVEVKDHGDPFYHKLSELLQGCR ### MCCNGCKAVVEVKDHGDPFYHKLSELLQGCR ### MCCNGCKAVVEVKDHGDPFYHKLSELLQGCR ### MCCNGCKAVVEVKDPDDPFYHKLSELLQGCR ### MCCNGCKAVVEVKDPPDPFYHKLSELLQGCR ### MCCNGCKAVVEVKDPDDPFYHKLSELLQGCR ### MCCNGCKAVAVARGCDDPPFYHKLSELLQGCR ### MCCNGCKAVAVARGCDDPPFYHKLSELLQGCR ### MCCNGCKAVAVARGCDDPFYHKLSELLQGCR ### MCCNGCKAVAVARGCDDPPFYHKLSELLQGCR ### MCCNGCKAVAVARGCDDPFYHKLSELLQGCR ### MCCNGCKAVAVARGCDDPFYHKLSELLQGCR ### MCCNGCKAVAVARGCDDPFYHKLSELLQGCR ### MCCNGCKAVAVARGCDDPFYHKLSELLQGCR ### MCCNGCKAVAVARGCDPFYHKLSELL	HD-3-BZ-IR	CSAVIA'MERCOLI COLI TITTICALICE COLI DOCUMENTO IL MOSS	S	•	35.6	1.0	1.0	
SECUNGATOR STATEMENT OF THE SELLOGE STATE	ng 2 u1rg	MI.CKROTHDDDFYHKI,SELAGCR	9	•	36.5	1.0	1.0	
37.7 36.4 37.6 1.0	np-3-nr-tr ug-3-ng-1P	SSKYVKATVGTPHDPFYHKLSELLOGS		•	35.9	1.0	1.0	
SCAAVKETHDPPDPFYHKLSELLHGC	np-3-A3-1N uR-3-H11-TR	GCAATAVATGNDNDFFYHKLSELLQGCR	37.7	9	37.6	1.0	1.0	
SCAAEKEVAGTARDPFYHKLSELMQSS CSVAVGDSGDPFYHKLSELLQGCR WQRNKQOIIGTPDDPFYHKLSELLEGS RSAAAKAVIGSPNDPFYHKLSELLEGS RSAAAKAVIGSPNDPFYHKLSELLQGG WLCDRDGRDEQPWDFYHKLSELLQGS GSVAAAKKTGSSDDPFYHKLSELLQGS GCKVDDE*ARSSDPFYHKLSELLQGCR GCKAVVEVKDGSDDPFYHKLSELLQGC GCKAVVEVKDGSDDPFYHKLSELLQGC CSTVTVSGSDDPFYHKLSELLQGC RSVTAKVEVGSDDPFYHKLSELLQGG GCKAVVENGGNDDPFYHKLSELLQGG CSTVTVSGSDDPFYHKLSELLQGCR GCKAVVAMGGNDDPFYHKLSELLQGCR GGCAVVTMAMGGNDDPFYHKLSELLQGCR GGCAVVTMAMGGNDDPFYHKLSELLQGCR GGCAVVTMAMGGNDDPFYHKLSELLQGCR GGCAVVAMGGNDDPFYHKLSELLQGCR GGCAVVAMGGNDDPFYHKLSELLQGCR GGCAVVAMGGNDDPFYHKLSELLQGCR GGCAVVAMGGNDDPFYHKLSELLQGCR GGCAVVAMGGNDDPFYHKLSELLQGCR GGCAVVAMGGNDDPFYHKLSELLQGCR GGCAVVAMGGNDDPFYHKLSELLQGCR GGCAVVAMGGNDDPFYHKLSELLQGCR GGCAVVANGGNDDPFYHKLSELLQGCR GGCAVVANGGNDDPFYHKLSELLQGCR GGCEVVANAGGNDDPFYHKLSELLQGCR GGCEVVANAGGNDDPFYHKLSELLQGCR GCCEVVANAGGNDDPFYHKLSELLQGCR GCCEVVANAGGNGDPFYHKLSELLQGCR GCCEVVANAGGNGTPFYHKLSELLQGCR GCCEVVANAGGNGTPFYHKLSELLQGCR GCCEVVANAGGNGTPFYHKLSELLQGCR GCCEVVANAGGNGTPFYHKLSELLQGCR GCCEVVANAGGNGTPFYHKLSELLQGCR GCCEVVANAGGNGTPFYHKLSELLQGCR GCCEVVANAGGNGTPFYHKLSELLQGCR GCCEVVANAGGNGTPFYHTLSELLQGCR GCCEVVANGGNGTPFYHTLSELLQGCR GCCEVVANGGNGTPFYHTLSELLQGCR GCCEVVANGGNGTPFYHTLSELLQGCR GCCEVVANGGNGTPFYHTLSELLQGCR GCCEVVANGGNGTPFYHTLSELLQGCR GCCEVANGGNGTPFYHTLSELLQGCR GCCEVANGGNGTPFYHTLSELLQGCR GCCEVANGGNGTPFYHTLSELLQGCR GCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	148-3-62-TR	GCAAVVKETHDPPDPFYHKLSELLHGC	•	36.5	37.2	1.0	1.0	
CSVAVGDEDEPFYHKLSELLQGCR WQRNKQQIIGTPDDFFYHKLSELLGGS RSAAAKAVIGSPUDPFYHKLSELLQGG WLCDRDGRDEQPWDFYHKLSELLQGG WLCDRDGRDEQPWDFYHKLSELLQGG GSVAAAKKTGSSDDFFYHKLSELLQGS GCAVTTWTMRSPADPFYHKLSELLQGG GCAVTTWTMRSPADPFYHKLSELLQGG GCKAVVEVWDGSDDFFYHKLSELLQGC GCKAVVEVWDGADPFYHKLSELLQGG GCKAVVEVGSDDFFYHKLSELLQGG GCKAVVEGTNDPFYHKLSELLQGG GCSTVTVSGSDDFFYHKLSELLQGG GCSTVTVSGSDDFFYHKLSELLQGG A1.1 41.4 41.9 1.1 CCDEKQRVTGGTNDPFYHKLSELLQGG GGAAVVAMGGNDDPFYHKLSELLQGG GGAAVVAMGGNDDPFYHKLSELLQGG GGAAVVAMGGNDDFFYHKLSELLQGG GGYTRAWAGGPDDFFYHKLSELLQGG GGYTRAWAGGPDDFFYHKLSELLQGG GGYTRAWAGGPDDFFYHKLSELLQGG GGYTRAWAGGPDDFFYHKLSELLQGG GGTTAEKVVGPPDDFFYHKLSELLQGG GGTTAEKVVGPPDDFFYHKLSELLQGG GGTTAEKVVGPPDDFFYHKLSELLQGG GGTTAEKVVGPPDDFFYHKLSELLQGG GGTTAEKVVGPPDDFFYHKLSELLQGG GGTTAEKVVGPPDDFFYHKLSELLQGG GGTTAEKVVGPPDDFFYHKLSELLQGG GGTTAEKVVGPPDDFFYHKLSELLQGG GGTTAEKVVGPPDDFFYHKLSELLQGG GGTTAEKVVGPPDFYHKLSELLQGG GGTTAEKVVGPPDFYHKLSELLQGG GGTTAEKVAGANAGDFFYHKLSELLQGG GGTTAEKVVGPPDFYHKLSELLQGG GGTTAEKVVGPPDFYHKLSELLQGG GGTTAEKVAGANAGDFFYHKLSELLQGG GGTTAEKVAGANAGDFFYHKLSELLQGG GGTTAEKVAGANAGDFFYHKLSELLQGG GGTTAEKVAGANAGDFFYHKLSELLQGG GGTTAEKVAGANAGDFFYHKLSELLQGG GGTTAEKVAGANAGDFFYHKLSELLQGG GGTTAEKVAGANAGGNDFFYHKLSELLQGG GGTTAETGGCT GTTATTTATTATTATTATTATTATTATTATTATTATTATT	inβ-3-cz in inβ-3-C8-IR	SCAAFKEVAGTARDPFYHKLSELMOSS		37.7	39.5	1.0	1.0	
MORNINGO GENERALEGIS 35.4 38.3 39.5 1.0	1113-3-6111-TP	CSVAVGDSGDPFYHKLSELLOGCR	•	38.2	39.1	1.0	1.0	
RSAAAKKTGSENDPFYHKLSELIQGG MLCDRDGRDEQPWDPFYHKLSELIQGS MLCDRDGRDEQPWDPFYHKLSELLQGS GSVAAAKKTGSSDDPFYHKLSELLQGS GCAVTTWTWRSPADPFYHKLSELLQGS GCAVTTWTWRSPADPFYHKLSELLQGC GCKAVVEVKDHGDDPFYHKLSELLQGC GCNTVAGGDDDPFYHKLSELLQGC GCTIABEKVVGPPDDPFYHKLSELLQGC GCTIABEKVGPPDDPFYHKLSELLQGC GCTIABEKVGPPDPFYHKLSELLQGC GCTIABEKVGPPDDPFYHKLSELLQGC GCTIABEKVGPPDPFYHKLSELLQGC GCTIABEKTATAGCC GCTIABEKTATAGCC GCTIABETTAGCC GCTIABETTAGCC GCTIABETTAGCC GCTIABETTAGCC GCTIABETTAGCC GCTIABETTAGCC GCTIA	11/2-3-D8-TR	WORNKOOTIGTPDDPFYHKLSELLEGS	•	38.3	39.5	1.0	1.0	
### MCCDRDGRDEQPWDPFYHKLSELVSCGR #### MCCDRDGRDEQPWDPFYHKLSELLQGS GSVAAAKKTGSSDDPFYHKLSELLQGS GCAVTTMTMRSPADPFYHKLSELLQGS GCKVDDE*ARSSDPFYHKLSELLQGC GCKAVVEVKDHGDDPFYHKLSELLQGC GCKAVVEVKDHGDDPFYHKLSELLQGC GCKAVVEVKDHGDDPFYHKLSELLQGC GCKAVVEVKDHGDDPFYHKLSELLQGC RSVTAKVEVGSDDPFYHKLSELLQGC RSVTAKVEVGSDDPFYHKLSELLQGC GSRQKIEVGTNDPFYHKLSELLQGC LCDEKQRVTGGTNDPFYHKLSELLQGC GGAAVVAMGGNDDPFYHKLSELLQGCR GGAAVVAMGGNDDPFYHKLSELLQGCR GGAAVVAMGGNDDPFYHKLSELLQGCR GGAAVVAMGGNDDPFYHKLSELLQGCR GGCEKVAVANGGNAGDPFYHKLSELLQGC GCEKVAVANGGNAGDPFYHKLSELLQGC GCEKVAVANGGNAGDPFYHKLSELLQGC GCEKVAVANGGNAGDPFYHKLSELLQGC GCEKVAVANGGNAGDPFYHKLSELLQGC GCEKVAVANGGNAGDPFYHKLSELLQGC GCEKVANANGGNAGDPFYHKLSELLQGC GCEKVANANGGNAGNAGDPFYHKLSELLQGC GCEKVANANGGNAGNAGDPFYHKLSELLQGC GCEKVANANGGNAGNAGNAGNAGNAGNAGNAGNAGNAGNAGNAGN	HB-3-B7-IR	RSAAAKAVIGSPNDPFYHKLSELIOGG		6	39.4	1.0	1.0	
GSVAAAKKTGŠSDDPFYHKLFELLQGS 39.0 39.8 41.1 1. GCAVTTMTMRSPADPFYHKLSELLQGR 40.1 40.4 41.1 1. GCKVDDE*ARSSDPFYHKLSELLQGC 40.8 40.7 40.7 1. GCKAVVEVKDHGDDFFYHKLSELLQGC 40.7 40.9 42.6 1. RSVTAKVEVGSDDPFYHKLSELLQGG 41.1 41.4 41.9 1. RSVTAKVEVGSDDPFYHKLSELLQGG 39.8 42.0 41.5 1. LCDEKQRVTGGTNDPFYHKLSELLQGCR 39.8 42.0 41.3 1. SCMVEGPNDDPFYHKLSELLQGCR 43.0 42.0 41.3 1. GGAAVVVAMGGNDDPFYHKLSELLQGCR 43.0 42.0 41.3 1. GCITAEKVVGPPDDPFYHKLSELLQGS 42.7 44.0 1. GCITAEKVVGPPDDPFYHKLSELLQGG 42.7 43.9 45.4 1. GCEKVVANAGABDPFYHKLSELLQGG 41.1 42.3 36.6 1. GCEKVVANAGABDPFYHKLSELLQGG 41.1 2.4 2.1 1.	HB-3-A12-TR	WICDRDGRDEOPWDFYHKLSELVSCGR	•	9	41.3	1.0	1.0	
GCAVTTMTMRSPADPFYHKLSELCQGR 40.1 40.4 41.1 1. GCKVDDE*ARSSDPFYHKLSELLLQGC 40.7 40.7 40.7 1. GCKAVVEVKDHGDDFFYHKLSELLQGC 40.7 40.9 42.6 1. RSVTAKVEVGSDRDFFYHKLSELLQGG 40.7 40.9 42.6 1. RSVTAKVEVGSDRDFYHKLSELLQGG 40.0 41.9 41.9 1. LCDEKQRVTGGTNDPFYHKLSELLQGCR 39.8 42.0 41.3 1. SCMVEGPNDPFYHKLSELLQGCR 40.0 42.6 43.3 1. GGAAVVVAMGGNDDPFYHKLSELLQGC 42.7 42.6 43.3 1. GGVIKAMKAGGPDDFFYHKLSELLQGC 42.7 44.0 1. GCITAEKVVGPPDDFFYHKLSELLQGC 41.1 42.3 36.6 1. GCEKVVANAGGNAGDPFYHKLSELLQGC 41.1 2.4 2.1 1.	HB-3-B4-TR	GSVAAAKKTGSSDDPFYHKLFELLQGS	•	39.8	41.1	1.0	1.0	
GCKAVDDE*ARSSDPFYHKLSELLKGCR 35.8 40.7 40.7 1. GCKAVVEVKDHGDDFFYHKLSELLQGC 40.8 40.7 39.5 1. CSTVTVSGSDDFFYHKLSELLQGS 40.7 40.9 42.6 1. RSVTAKVEVGSDRDPFYHKLSELLQGS 40.0 41.9 41.9 1. GSRRQKI EVGTNDPFYHKLSELLQGCR 39.8 42.0 41.3 1. SCMVEGPNDPFYHKLSELLQGCR 40.7 42.6 43.3 1. GGAAVVVAMGGNDDFFYHKLSELLQGG 42.7 44.0 1. GGVI KAMKAGGPDDFFYHKLSELLQGS 42.7 43.9 45.4 1. GCI IAEKVVGPPDDFFYHKLSELLDGG 41.1 42.3 36.6 1. GCEKVVANAGGNAGDPFYHKLSELLLQGC 41.1 42.3 36.6 1. GCEKVVANAGGNAGDPFYHKLSELLLQGC 41.1 2.4 2.1 1.	πβ 3 21 IN	GCAVTTMTMRSPADPFYHKLSELCQGR	40.1	40.4	41.1	1.0	1.0	
GCKAVVEVKDHGDDPFYHKLSELLQGC 40.8 40.7 39.5 1. CSTVTVSGSDDPFYHKLSELLQGS 40.7 40.9 42.6 1. RSVTAKVEVGSDRDPFYHKLSELLQGG 40.0 41.4 41.9 1. LCDEKQRVTGGTNDPFYHKLSELLQGGR 39.8 42.0 41.6 1. SCMVEGPNDPFYHKLSELLQGCR 40.7 42.6 43.3 1. GGAAVVVAMGGNDDPFYHKLSELLQGG 43.0 42.7 44.0 1. GGVIKAMKAGGPDDPFYHKLSELLQGS 42.7 43.9 45.4 1. GCTIAEKVVGPPDDPFYHKLSELLQGS 41.1 42.3 36.6 1. GCEKVVANGGNAGDPFYHKLSELLQGS 41.1 42.3 36.6 1. GCEKVVANGGNAGDPFYHKLSELLQGS 41.1 2.4 2.1 1.	HB-3-E12-IR	GCKVDDE*ARSSDPFYHKLSELLKGCR	•	40.7	40.7	1.0	1.0	
CSTVTVSGSDDPFYHKLSELLQGG RSVTAKVEVGSDDPFYHKLSELLQGG GSRRQKIEVGTPNDPFYHKLSELLQGG LCDEKQRVTGGTNDPFYHKLSELLQGG LCDEKQRVTGGTNDPFYHKLSELLQGCR SCMVEGPNDDPFYHKLSELLQGCR GGAAVVVAMGGNDDPFYHKLFELMQGG GGVIKAMKAGGPDDPFYHKLSELLQGS GCIIAEKVVGPPDDPFYHKLSELLQGS GCIIAEKVVGPPDDPFYHKLSELLQGC GCEKVVAVAGNAGDPFYHKLSELLQGC GCEKVVAVAGNAGDPFYHKLSELLQGC GCEKVVAVAGNAGDPFYHKLSELLQGC 4.1 2.4 2.1 1.1	HB-3-B8-IR	GCKAVVEVKDHGDDPFYHKLSELLQGC		40.7	39.5	1.0	1.0	
RSVTAKVEVGSDRDPFYHKLSELLQGS GSRRQKIEVGTPNDPFYHKLSELLQGG LCDEKQRVTGGTNDPFYHKLSELLQGG LCDEKQRVTGGTNDPFYHKLSELLQGCR SCMVEGPNDDPFYHKLSELLQGCR GGAAVVVAMGGNDDPFYHKLSELLQGGS GGVIKAMKAGGPDDPFYHKLSELLQGS GCVIKAMKAGGPDDPFYHKLSELLQGS GCIIAEKVVGPPDDPFYHKLSELLQGC GCIIAEKVVGPPDDPFYHKLSELLQGC GCIEKVANANGNAGNPFYHKLSELLQGC 4.1 2.4 2.1 1.1 1.1	HB-3-C5-IR	CSTVTVSGSDDPFYHKLSELLQGC	•		2	1.0	1.0	
GSRRQKIEVGTPNDPFYHKLSELLGGG 40.0 41.9 41.6 1. LCDEKQRVTGGTNDPFYHKLSELLGGCR 39.8 42.0 41.3 1. SCMVEGPNDDPFYHKLSELLQGCR 40.7 42.6 43.3 1. GGAAVVVAMGGNDDPFYHKLSELLQGS 42.7 44.0 1. GGVIKAMKAGGPDDPFYHKLSELLQGS 42.7 43.9 45.4 1. GCIIAEKVVGPPDDPFYHKLSELLQGS 41.1 42.3 36.6 1. GCEKVVANAGNAGDPFYHKLSELLQGS 41.1 2.4 2.1 1.	3HB-3-A2-IR	RSVTAKVEVGSDRDPFYHKLSELLQGS	41.1		т Т	1.0	1.0	
LCDEKQRVTGGTNDPFYHKLSELTGGCR 39.8 42.0 41.3 1. SCMVEGPNDDPFYHKLSELLQGCR 40.7 42.6 43.3 1. GGAAVVVAMGGNDDPFYHKLFELMQGG 43.0 42.7 44.0 1. GGVIKAMKAGGPDDPFYHKLSELLQGS 42.7 43.9 45.4 1. GCIIAEKVVGPPDDPFYHKLSELLQGG 41.1 42.3 36.6 1. GCEKVVANAGNAGDPFYHKLSELLQGG 4.1 2.4 2.1 1.	HB-3-A8-IR	GSRROKIEVGTPNDPFYHKLSELLQGG	•	41.9	1.	1.0	1.0	
SCMVEGPNDDPFYHKLSELLLQGCR 40.7 42.6 43.3 1. GGAAVVVAMGGNDDPFYHKLFELMQGG 43.0 42.7 44.0 1. GGVIKAMKAGGPDDFFYHKLSELLDQGS 42.7 43.9 45.4 1. GCIIAEKVVGPPDDPFYHKLSELLDCG 41.1 42.3 36.6 1. GCEKVVAVAGNAGDPFYHKLSELLQGC 4.1 2.4 2.1 1.	HB-3-C12-IR	LCDEKORVIGGINDPFYHKLSELIGGCR		42.0	41.3	1.0	1.0	
GGAAVVVAMGGNDDPFYHKLFELMQGG 43.0 42.7 44.0 1. GGVIKAMKAGGPDDPFYHKLSELLQGS 42.7 43.9 45.4 1. GCIIAEKVVGPPDDPFYHKLSELLQGC 41.1 42.3 36.6 1. GCEKVVAVAGNAGDPFYHKLSELLQGC 4.1 2.4 2.1 1.	HB-3-B11-TR	SCMVEGPNDDPFYHKLSELLQGCR	•	2	43.3	1.0	1.0	
GGVIKAMKAGGPDDPFYHKLSELLQGS GGVIKAMKAGGPDDPFYHKLSELLQGS 41.1 42.3 36.6 1. GCIRAEKVVAVAGNAGDPFYHKLSELLQGC 4.1 2.4 2.1 1.	HB-3-C3-TR	GGAAVVVAMGGNDDPFYHKLFELMQGG		42.7	4.	1.0	1.0	
GCIIAEKVVGPPDDPFYHKLSELLDCG 41.1 42.3 36.6 1. GCEKVVAVAGNAGDPFYHKLSELLQGC 4.1 2.4 2.1 1.	SHR-3-R5-TR	GGVIKAMKAGGPDDPFYHKLSELLQGS	2	Э.	5.	1.0	1.0	
GCEKVVAVAGNAGDPFYHKLSELLQGC 4.1 2.4 2.	5HB-3-C4-IR	GCIIAEKVVGPPDDPFYHKLSELLDCG	41.1			1.2	6.0	
ACCOUNT OF THE POST OF THE POS	SHR-3-G7-IR	GCEKVVAVAGNAGDPFYHKLSELLQGC	4.1		2.1	1.1	6.0	
GSVMIVI BIMAGADD PF I I NEDEBLINGGO	Bomp 3 CT IN	GSVMTVTEMAGADDPFYHKLSELLQGGR	29.2	30.6	28.2	1.1	6.0	

		Ratios ove	Ratios over Background	pun	Comparisons	isons
Clone	Sequence	E-Tag	IGFSK	¥	IGFK/IK IK/IGFK	ואוסרא -
Design	OOUUUUUUUJJJJDFFYHKLSELXXOO	:	;	ŀ		
25.6. 25.4.12_TB	RCEAKEAKIGSAGDPFYHKLSELMOGSR	33.6	32.0	29.5	1.1	٥.0
Bonp-3-in-c-dnoa	COFFYXXMANSSADPFYHKISELCOGSR	30.1	34.3	30.5	1.1	6.0
Borp-3-G10-1A	CCBLVVTTCCDNDPFYHKI,SELLOGCR	37.1	35.3	32.4	1.1	6.0
Bonp-3-rio-in	SS.I.LIRR.THVRQUAHANASATA	39.9	38.9	35.5	1.1	6.0
B6HD-3-D5-IK	SATGENZY VOSITATE TIMESTALIZATE OF THE CONTRACT OF THE CONTRAC	39.5	40.0	37.1	1.1	6.0
B6HD-3-B12-IK	GCNEVVELENTATION OF THE CONTRACT OF THE CONTRA	40.4	41.5	39.1	1.1	6.0
B6HD-3-D2-IR	MALLING GOODDEN TO THE SELECTION OF THE	41.1	44.6	36.6	1.2	0.8
B6HD-3-DI-IR	WCDZNEIVVONODELI IIII EEE TOOLE	34.3	36.4	24.1	1.5	0.7
B6HD-3-G6-IR	CSVVMTFEKNDRDDPFYHKLSELLOGC	38.1	30.9	18.4	1.7	9.0
B6HB-3-A/-IN B6HB-3-B10-IR	GGEARRROOVGTANDPFYHKLSELAFGGR	32.3	36.5	22.8	1.6	9.0
B6H8-3-B9-IR	GCAVTAITINGTSDPFYHKLSELCOGS	38.6	38.5	20.8	1.9	0.5
B6HR-3-D6-IR	GSKVKAMAVGTSDDPFYHKLSELVOGR	35.9	36.0	15.6	2.3	0.4
B6Hβ-3-C7-IR	RCKGIKAHSDNDPFYHKLSELCQGG	38.3	38.0	9.9	5.8	0.2

Clare	у дения в в в в в в в в в в в в в в в в в в в	Ratios ove	Ratios over Background E-Tag IGFsR II	ınd IR	Comparisons IGFR/IR IR/IGFR	ons VIGFR
Design) ! !	1 1 1	!	:	
REH-3-F1-IGFR	RRVAAVA?KDATGDPFYHKLSELLRSG	20.0	30.8	ŀ	1 1	:
BEH-3-D4-IGFR	RSTMKEKIEGDGNDPFYHKLSELLKSG	19.0	27.6	1	1	1
R6H-3-G3-IGFR	GGAVIVTAARRGSDPFYHKLSELVGRG	14.2	25.2	l t	1	•
BGH-3-F2-IGFR	SREAVEVTMARGSDPFYHKLSELVWGS	12.5	24.8	:	!	:
B6H-3-D1-IGFR	RSTTMVKAVPPPRDPFYHKLSELL*GG	20.0	24.2	1	1	1
B6H-3-A3-IGFR	GRTEEVVVVGTRRDPFYHKLSELLASG	14.2	22.8	1	I I	
B6H-3-A4-IGFR	RRMAGWQ*TSSSDPFYHKLSELVSGS	13.0	22.8	1	i I	:
B6H-3-B4-IGFR	SRKEVTEMVGGPSDPFYHKLSELMGSG	10.2	22.8	l I	1	1
B6H-4-D11-IGFR	RGTAKQRKSSDP*DPFYHKLSELIYGS	14.0	22.5	ı i	1	i t
B6H-3-G2-IGFR	GGVVAVVAAGRRDDPFYHKLSELVSGR	15.2	22.5	!	i i	1
B6H-3-B2-IGFR	SR.MAMVEVGNPGDPFYHKLSELLGS	14.5	21.9	t ŧ	!	1
B6H-3-E2-IGFR	RRVTAVI EVDGADDPFYHKL?ELLSGG	11.6	21.8	;	1	l !
B6H-3-B1-IGFR	RSVIAN???G?NADPFYHKLSELISSG	15.9	21.7	!	1 1	:
B6H-4-G3-IGFR	RGVVIETTKDPGADPFYHKLSELLFGR	19.1	21.4	:	1	:
B6H-4-F9-IGFR	RRTTVMETVGGRDDPFYHKLSELLHRG	11.3	20.9	1	I I	1 1
B6H-3-E3-IGFR	GRVVVAAAVRPDDDPFYHKLSELVAGR	14.2	20.8	E I	1 1	1
B6H-3-E1-IGFR	RGVATVVVANHHSDPFYHKLSELVLRG	20.0	20.6	! ;	;	!
B6H-4-F3-IGFR	RRKMATEIMRSDADPFYHKLSELLGGS	12.5	20.3	!	1	• •
B6H-3-D2-IGFR	GGKTAVEVTSPASDPFYHKLSELLLRG	12.1	19.3	t t	1	!!
B6H-4-A9-IGFR	RREKKVKVTTTDNDPFYHKLSELVFGG	14.1	19.2	1	!	1
B6H-4-E6-IGFR	SSAIIMVAADRADDPFYHKLSELLWGS	12.5	19.2	i	î !	1
B6H-4-C3-IGFR	RREVAIVAAGAGGDPFYHKLSELLSRG	23.6	18.9	!	:	l I
B6H-3-C2-IGFR	RRMVMEAAENHADDPFYHKLSELLWRD	16.2	18.5	1	\$ 1	! !
B6H-3-C4-IGFR	GRKMEIVAIRGAHDPFYHKLSELL*GR	16.8	17.2	i 1	1	1 1
B6H-3-C3-IGFR	CCIAMVEMAAGGGDPFYHKLSELLSGR	14.6	17.1	i I	i 1	1
B6H-3-B3-IGFR	RGAQSPDPFYHKLSELAFGS	0.6	16.8	1	i I	1
B6H-4-H3-IGFR	RKTAMVVIGDASDPFYHKLSELAFGS	10.1	16.6	i I	1 1	!
B6H-3-H3-IGFR	GSVITKAMKADGDDPFYHKLSELL*GG	14.2	16.4	!	!	1

		Ratios ove	Ratios over Background	pun	Comparisons	isons
Clone	Sequence	E-Tag	IGFsR	¥	IGFR/IR IR/IGFR	IR/IGFR
Deaton	OOUTUUUUJJJJDPFYHKLSELXXOO	1 1	1 1 1	t t	1 1	
DCCLS:	GGVKAAAAERDDSDPFYHKLSELLFGS	15.1	16.4	!	i t	i I
D6H-4-D6-1G1N D6H-4-D6-1GFP	CCEMVKTIEHGGNDPFYHKLSELVFGR	12.6	15.6	1	1	1
B6H-4-D8-13FA B6H-4-F10-1GFR	GGAKVAVVVDHGDDPFYHKLSELLRGS	10.2	15.1	t i	1	1 1
D6H-4-E10-1011 D6H-4-E10-10FR	RGKTKMAMAAGGNRDPFYHKLSELI FGN	12.3	14.8	t I	1	1
B6H-4-L3 1GFR B6H-4-R2-IGFR	GGMATKIVTAPGHDPFYHKLSELLFGG	9.9	11.8	r I	! 1	!
BOII 4 DZ ICIN	SGEGEMAMPGPDDPFYHKLSELIGSRA	8.2	11.6	1	l ł	1
BOH 3 - A2 - IGFR	GGMAEVVVVGPPRDPFYHKLSELVGGG	10.9	9.6	1	1	! !
B6H-3-H2-IGFR	GGEVKVMVADGSTDPFYHKLSELLGRT	5.9	9.6	1	i i	!
BGH-4-A1-IGFR	SCVMVETVAGRNRDPFYHKLSELVGGC	4.4	9.5	t t	1	l 1
B6H-3-H1-IGFR	RRW*KVPGAADPFYHKLSELLGRSA	7.2	8.7	1	1	1
B6H-4-C2-IGFR	GGVEATEVEHADGDPFYHKLSELVGRS	6.7	8.6	1 1	1 1	į į
5H-4-H9-IGFR	RGVEVAVITHGPPDPFYHKLSELLRGA	12.3	8.4	I I	I f	! 1
B6H-4-B7-IGFR	SGTVTVIAMSGTDDPFYHKLSELLSRS	6.4	8.2	1	!	1
B6H-4-A7-IGFR	GRTAVVKEASPAHDPFYHKLSELLLRG	7.6	8.1	!	1	i i
B6H-4-B3-IGFR	RGAI GNAAVGNRSDPFYHKLSELISRG	4.4	7.8	! !	1	1
B6H-4-B4-IGFR	GGMIKTAMEHDTRDPFYHKLSELLRGG	5.2	7.4	t t	t 1	1
B6H-4-E1-IGFR	GCAEVEEVAGAGHDPFYHKLSELCAGG	3.6	7.1	ı	! ŧ	t i
B6H-3-C1-IGFR	SSVVVVEVVDARRDPFYHKLSELV?SG	5.7	4.6	1	1	:
B6H-4-A3-IGFR	GRKKAVATMTDGGDPFYHKLSELILRS	4.4	4.2	1	1	1
B6H-4-H10-IGFR	RGETEMAVADTDDDPFYHKLSELLGRG	4.4	3.5	1	:	i i
B6H-3-G1-IGFR	GORDPFYHKLSELMGRGA	2.4	2.9	i i	i	t I

		Ratios ove	Ratios over Background	pun	Comparisons	isons	
Clone	Sequence	E-Tag	IGFSR	R	IGFR/IR	IR/IGFR	
Design	EHWNTVDPFYHKLSELLRESG	1	!		:	i i	
B6C-3-C7-IR	EHWNTVDPFYFTLFE*LRESG	31.7	2.1	20.0	0.1	9.4	
B6C-4-F2-IR	EHWNTVDPFYNOLWEWLRESG	8.2	1.8	4.3	0.4	2.4	
B6C-3-A2-TR	EHWNTVDPFYHOLSEWLRESG	34.9	18.1	36.0	0.5	2.0	
B6C-4-H11-TR		37.1	28.2	38.6	0.7	1.4	
B6C-4-H4-TR	EHWNTVDPFYROLSEWLRESG	39.5	28.3	39.4	0.7	1.4	
B6C-3-A11-IR	EHWNTVDPFYHYFOELLRESG	25.4	25.9	34.2	0.8	1.3	
B6C-3-D9-TR	EHWNTVDPFYHOMYEWLRESG	35.7	30.3	37.2	0.8	1.2	
B6C-4-G4-TB	EHWNTVDPFYROLYEWLRESG	35.3	31.0	38.4	0.8	1.2	
B6C-3-C6-IR	EHWNTVDLFYYGLOELLRESG	33.3	33.9	35.9	0.9	1.1	
B6C-3-D8-IR	EHWNTVDPFYH*ISELLRESG	34.5	34.7	37.1	0.9	1.1	
B6C-4-G7-IR	EHWNTVDPFYOFFAELLRESG	35.9	36.9	38.9	0.9	1.1	
B6C-3-C8-IR	EH*NTVDPFYEGLLELLRESG	35.6	37.2	39.6	6.0	1.1	
B6C-3-D6-IR	EH*NTVDPFYQGLFELLRESG	37.6	37.6	40.2	6.0	1.1	
B6C-3-C10-IR	EHWNTVDPFYQYFSELLRESG	35.3	36.4	40.6	0.9	1.1	
B6C-3-B3-IR	$\mathtt{EHWNTVDPFY}$ GLQTLLRESG	38.3	38.7	40.8	0.9	1.1	
B6C-3-B1-IR	EHWNTVDPFYQALFELLRESG	37.8	38.9	41.2	0.9	1.1	
B6C-4-F6-IR	EHWNTVDPFYD*MRNLLRESG	35.8	36.8	38.7	1.0	1.1	
B6C-3-B11-IR	EHWNTVDPFYNLLQELLRESG	36.3	37.0	38.8	1.0	1.1	
B6C-3-B8-IR	EHWNTVDPFYDGLRQLLRESG	37.2	39.2	41.2	1.0	1.1	
B6C-3-C12-IR	EHWNTVDPFYGKLQELLRESG	28.3	28.7	28.9	1.0	1.0	
B6C-3-C2-IR	EHWNTVDPFYQQLFELLRESG	34.1	34.7	33.8	1.0	1.0	
B6C-3-D5-IR	EHWNTVDPFYLMLQQLLRESG	33.9	35.3	34.1	1.0	1.0	
B6C-4-F7-IR	EH*NTVDPFYHKLYELLRESG	34.9	34.7	34.2	1.0	1.0	
B6C-4-H2-IR	EHWNTVDPFYH*MSNLLRESG	35.4	35.8	35.8	1.0	1.0	
B6C-3-B12-IR	EHWNTVDPFYY*MSELLRESG	33.6	35.2	36.0	1.0	1.0	
B6C-3-A12-IR	EHWNTVDPFYQLLFELLRESG	33.1	37.0	36.2	1.0	1.0	
B6C-4-E9-IR	EHWNTVDPFYQRMFELLRESG	36.1	36.0	36.2	1.0	1.0	
B6C-4-E8-IR	EHWNTVDPFYQGLWELLRESG	34.2	35.0	36.6	1.0	1.0	

PFYHKLSELLRESG PFYD* ISELLRESG PFYHLLQELLRESG PFYHMLQELLRESG PFYHMLQELLRESG PFYHYLQDLLRESG PFYGGLSELLRESG PFYQGLSELLRESG PFYQGLSELLRESG PFYQGLSELLRESG PFYQKLQELLRESG PFYQKLQELLRESG PFYLKMQDLLRESG PFYLKMQDLLRESG PFYLKMQDLLRESG PFYLKMQDLLRESG PFYLKMQDLLRESG PFYLKLQELLRESG PFYHYMSQLLRESG PFYHYMSQLLRESG PFYHYMSQLLRESG PFYAKLQELLRESG PFYHGLYELLRESG
EHWNTVDPFYD* 1SELLKESG EHWNTVDPFYHLLQELLRESG EHWNTVDPFYHLLQELLRESG EHWNTVDPFYHYLQDLLRESG EHWNTVDPFYLGLQELLRESG EHWNTVDPFYLGLQELLRESG EHWNTVDPFYLMLQELLRESG EHWNTVDPFYLMLQELLRESG EHWNTVDPFYLMLQELLRESG EHWNTVDPFYLMLQELLRESG EHWNTVDPFYLKLQELLRESG EHWNTVDPFYLKLQELLRESG EHWNTVDPFYLKLQELLRESG EHWNTVDPFYLKLGELLRESG EHWNTVDPFYLKLGELLRESG EHWNTVDPFYLKLGELLRESG EHWNTVDPFYLKLGELLRESG EHWNTVDPFYLKLGELLRESG EHWNTVDPFYLKLGELLRESG EHWNTVDPFYLKLGELLRESG EHWNTVDPFYLGLELLRESG EHWNTVDPFYLKLGELLRESG EHWNTVDPFYLGLELLRESG EHWNTVDPFYLGLELLRESG EHWNTVDPFYLGLELLRESG EHWNTVDPFYLGLELLRESG EHWNTVDPFYLGLELLRESG

FIGURE 2J (Con't)

		Ratios ove	Satios over Background		Comparisons	sons
Clone		E-Tag	IGFsR IR		IGFR/IR IR/IGFR	IR/IGFR
Design	EHWNTVDPFYHKLSELLRESG	1	1	!	1	1 1
DESTURED DECEMBER 18	FHWNTVDPFYOKI,FELLRESG	36.3	36.9	34.3	1.1	6.0
BCC-4-F4 IN	FHWNTVDPFYH*LAFLI.RESG	8.5	10.3	9.0	1.2	6.0
B6C-3-D2-IN	HWNTVDDFYH*I.NEII.RESG	26.5	26.5 30.7 16.8	16.8	1.8	1.8 0.5
DCC-3 AL IN	FHWNTVDPFYHKLOELLRESG	33.4	33.0	15.0	2.2	0.5
B6C-3-A4-IR	EHWNTVDPFYRRLÖELLRESG	33.6		13.5	2.4	0.4

Colone			Ratios ove	Ratios over Background	pun	Comparisons	risons
######################################	Clone	Sequence	E-1 ag	IGFSK	<u> </u>	IGFKIR	NIGER.
EHWNTVDPFYHKLEELLRESGA EHWNTVDPFYHKLEELLRESGA EHWNTVDPFYHKLEELLRESGA EHWNTVDPFYKLSELLRESGA EHWNTVDPFYOKLSELLRESGA EHWNTVDPFYOKLSELLRESGA EHWNTVDPFYHRLSELLRESGA EHWNTVDPFYHRLSELLRESGA EHWNTVDPFYHRLSELRESGA EHWNTVDPFYHRLSELRESGA EHWNTVDPFYHRLSELRESGA EHWNTVDPFYAKLSELRESGA EHWNTVDPFYAKLSELRESGA EHWNTVDPFYAKLSALRESGA EHWNTVDPFYAKLSALRESGA EHWNTVDPFYAKLSALRESGA EHWNTVDPFYAKLSALRESGA EHWNTVDPFYAKLSGALRESGA EHWNTVDPFYAKLSGALRESGA EHWNTVDPFYAKLSGALRESGA EHWNTVDPFYAKLSGALRESGA EHWNTVDPFYAKLSGALRESGA EHWNTVDPFYAKLSELRESGA EHWNTVDPFYAKLSGA EHWNTVDPFYAKLSGA EHWNTVAFF SOW 13.6 EHWNTVAFF SOW 10.1 10.1 EHWNTVAFF SOW 10.1	Design		1	!	1 1	! !	:
EHWNTVDPFYHKLEELLRESGA EHWNTVDPFYGKLSELLRESGA EHWNTVDPFYQKLSELLRESGA EHWNTVDPFYQKLSELLRESGA EHWNTVDPFYQKLSELLRESGA EHWNTVDPFYGRLFELLRESGA EHWNTVDPFYHLXELLRESGA EHWNTVDPFYHLXELLRESGA EHWNTVDPFYHLXELLRESGA EHWNTVDPFYHLXELLRESGA EHWNTVDPFYHLXELLRESGA EHWNTVDPFYHLXELLRESGA EHWNTVDPFYHLXELLRESGA EHWNTVDPFYHLLAELLRESGA EHWNTVDPFYHLLAELLRESGA EHWNTVDPFYHLLAELLRESGA EHWNTVDPFYHLLAELLRESGA EHWNTVDPFYHLLAELLRESGA EHWNTVDPFYHKLSELLRESGA EHWNTVDPFYHKLSELLRESGA EHWNTVDPFYHKLSELRESGA EHWNTVDPFYHKLGELRESGA EHWNTVDPFYHKGA EHWNTVDPFYHKGA E	36C-3-C4-IGFR			23.1	1	:	1
EHWNTVDPFYUKLYELLRESGA EHWNTVDPFYQKLSELLRESGA EHWNTVDPFYQKLSELLRESGA EHWNTVDPFYRLSELLRESGA EHWNTVDPFYRGLQELLRESGA EHWNTVDPFYRHLSELLRESGA EHWNTVDPFYRHLSELLRESGA EHWNTVDPFYRHLSELLRESGA EHWNTVDPFYRKLSELLRESGA EHWNTVDPFYRKLSELLRESGA EHWNTVDPFYRKLSELLRESGA EHWNTVDPFYRKLSALLRESGA EHWNTVDPFYRKLSALLRESGA EHWNTVDPFYRKLSALLRESGA EHWNTVDPFYRKLSALLRESGA EHWNTVDPFYRKLSALLRESGA EHWNTVDPFYRKLSALLRESGA EHWNTVDPFYRKLSGALRESGA EHWNTVDPFYRKLSGALRESGA EHWNTVDPFYRKLSGALRESGA EHWNTVDPFYRKLSELLRESGA EHWNTVDPFYRKLSELLRESGA EHWNTVDPFYRKLSELLRESGA EHWNTVDPFYRKLSELLRESGA EHWNTVDPFYRKLSELLRESGA EHWNTVDPFYRKLSELLRESGA EHWNTVDPFYRKLSELLRESGA EHWNTVDPFYRKLSGALRESGA EHWNTVDFYRKLSELLRESGA EHWNTVDFYRKLSGALRESGA EHWNTVDFYRKLSELLRESGA EHWNTVDFYRKLSGALRESGA EHWNTVDFYRKLSGA EHWNTVDFYRKLSGA EHWNTVDFYRKGALRESGA EHWNTVDFYRKLSGA EHWNTVDFYRKGALRESGA	36C-3-F5-IGFR	EHWNTVDPFYHKLEELLRESGA	26.5	22.6	1	1	l 1
EHWNTVDPFYQKLSELLRESGA EHWNTVDPFYQKLSELLPESGA EHWNTVDPFYQRLFELLRESGA EHWNTVDPFYRHLSELLRESGA EHWNTVDPFYHRLSELLRESGA EHWNTVDPFYHRLSELLRESGA EHWNTVDPFYNKLSELLRESGA EHWNTVDPFYNKLSELLRESGA EHWNTVDPFYAKLSALLRESGA EHWNTVDPFYAKLSELLRESGA EHWNTVDPFYAKLSELLRESGA EHWNTVDPFYAKLSELLRESGA EHWNTVDPFYHKLSELLRESGA EHWNTVDPFYHKLSELRESGA EHWNTVDPFYHKLSELLRESGA EHWNTVDPFYHKLSELRESGA EHWNTVDFYHLLRESGA EHWNTVDFYHLLRESGA EHWNTVDFYHLRESGA EHWNTVDFYHLRESGA EHWNTVDFFYHKLSELLRESGA EHWNTVDFFLURRSGA EHWNTVGFFGA*-LLRESGA EHWNTGFFGA*-LLRESGA EHWNTGFFGA*-LLRESGA EHWNTGFFGA*-LLRESGA EHWNTGFFGA*-LLRESGA EHWNTGFFGA*-LLRESGA EHWNTGFF	B6C-3-D4-IGFR	EHWNTVDPFYHKLYELLRESGA	•	22.1	1	1	1
EHWITUDPFYLKLSELL?ESGA EHWNTUDPFYQRLFELLRESGA EHWNTUDPFYQRLFELLRESGA EHWNTUDPFYHGLQELLRESGA EHWNTUDPFYHRLSELLRESGA EHWNTUDPFYHRLSELLRESGA EHWNTUDPFYHRLSELLRESGA EHWNTUDPFYAKLSALLRESGA EHWNTUDPFYAKLSALLRESGA EHWNTUDPFYAKLSALLRESGA EHWNTUDPFYAKLSALLRESGA EHWNTUDPFYAKLSALLRESGA EHWNTUDPFYAKLSGALLRESGA EHWNTUDPFYAKLSGALLRESGA EHWNTUDPFYAKLSELLRESGA EHWNTUDPFYAKLSELLRESGA EHWNTUDPFYAKLSELLRESGA EHWNTUDPFYAKLSELLRESGA EHWNTUDPFYHKLSELLRESGA EHWNTUDPFYHKLSELLRESGA EHWNTUDPFYHKLSELLRESGA EHWNTUDPFYHKLAELLRESGA EHWNTUDPFYHKLQELLRESGA EHWNTUDPFYHKLAELLRESGA EHRONTUDPFYHKLAELLRESGA EHRONTUDPFYHKLAELLRESGA EHRONTUDPFYHKLAELL	BGC 3 - A3 - IGFR	EHWNTVDPFYOKLSELLRESGA	29.7		1	l t	l i
EHWNTVDPFYQRLFELLRESGA EHWNTVDPFYHGLQELLRESGA EHWNTVDPFYHRLSELLRESGA EHWNTVDPFYHRLSELLRESGA EHWNTVDPFYHRLSELLRESGA EHWNTVDPFYQKLSELLRESGA EHWNTVDPFYQKLSELLRESGA EHWNTVDPFYARLSALLRESGA EHWNTVDPFYHILAELLRESGA EHWNTVDPFYHILAELLRESGA EHWNTVDPFYHRLSELLRESGA EHWNTVDPFYHRLSELLRESGA EHWNTVDPFYHRLSELLRESGA EHWNTVDPFYHRLSELLRESGA EHWNTVDPFYHRLSELLRESGA EHWNTVDPFYHRLSELLRESGA EHWNTVDPFYHRLSELLRESGA EHWNTVDPFYHRLAELLRESGA EHWNTVDPFYHRLAELLRESGA EHWNTVDPFYHRLAELLRESGA EHWNTVDPFYHRLAELLRESGA EHWNTVDPFYHRLAELLRESGA EHWNTVDPFYHRLAELLRESGA EHWNTVDPFYHRLAELLRESGA EHWNTVDPFYHRLGELLRESGA EHWNTVDFYHRLGELLRESGA EHWNTVDFYHRLGELLRESGA EHWNTVDFYHRLGELLRESGA EH	BGC 3 - CO - TGFR	EHW?TVDPFYLKLSELL?ESGA	•	20.5	1	i	i 1
EHWNTVDPFYHGLQELLRESGA EHWNTVDPFYHRLSELLRESGA EHWNTVDPFYHRLSELLRESGA EHWNTVDPFYNKLSELLRESGA EHWNTVDPFYQKLSELLRESGA EHWNTVDPFYQKLSELLRESGA EHWNTVDPFYAKLSALLRESGA EHWNTVDPFYAKLSALLRESGA EHWNTVDPFYHILAELLRESGA EHWNTVDPFYPRICAELLRESGA EHWNTVDPFYPRICAELLRESGA EHWNTVDPFYPRICAELLRESGA EHWNTVDPFYPRICAELRESGA EHWNTVDPFYPRICAELLRESGA EHWNTVDPFYRKLSELLRESGA EHWNTVDPFYRKLSELLRESGA EHWNTVDPFYRKLAELLRESGA EHWNTVDPFYRKLAELLRESGA EHWNTVDPFYRKLAELLRESGA EHWNTVDPFYRKLAELLRESGA EHWNTVDPFYRKLAELLRESGA EHWNTVDPFYRKLGELLRESGA EHWNTVDFYRKLGELLRESGA EHWNTVDFYRKLGELLRESGA EHWNTVDFYRKLSTGA EHWNTVDFYRKLSTGA EHWNTVDFYRKLSTGA EHWNTVDFYRKLSTGA EHWNTVDFYRKLSTGA EHWN	BGC 3 - CB - IGFR	EHWNTVDPFYORLFELLRESGA	30.5	•	1	t i	1
### ### ### ### ### ### ### ### ### ##	B6C-3-A8-IGFR	EHWNTVDPFYHGLQELLRESGA	29.7	19.7	\$ †	l l	!
EHWNTVDPFYNKLSELLRESGA EHWNTVDPFYNKLSELLRESGA EHWNTVDPFYQKLSELLRESGA EHWNTVDPFYAKLSALLRESGA EHWNTVDPFYARLSALLRESGA EHWNTVDPFYHILAELLRESGA EHWNTVDPFYHILAELLRESGA EHWNTVDPFYDRLTELLRESGA EHWNTVDPFYDRLTELLRESGA EHWNTVDPFYHKLSELLRESGA EHWNTVDFYHKLSELLRESGA EHWNTVDFYHKLSFLLRESGA EHWNTVDFYHKLSFLLRESGA EHWNTVDFYHKLSFLLRESGA EHWNTVDFYLLRESGA EHWNTVBYLLRESGA EHWNTVBYLLRESGA EHWNTVDFYLLRESGA EHWNTVBY	B6C-3-A2-IGFR	EHWNTVDPFYHRLSELLRESGA	•	19.0	t t	1	i i
### EHWNTVDPFYNKLSELLRESGA ###	B6C-3-A9-IGFR	EHWNTVDPFYHNLYELLRESGA	33.0	18.3	!	t t	1
EHWNTVDPFYQKLSELLRESGA EHWNTVDPFYAKLSALLRESGA EHWNTVDPFYARLSALLRESGA EHWNTVDPFYARLSALLRESGA EHWNTVDPFYHILAELLRESGA EHWNTVDPFYHILAELLRESGA EHWNTVDPFYPTALLELRESGA EHWNTVDPFYPTALLRESGA EHWNTVDPFYHKLSELLRESGA EHWNTVDPFYHKLSELLRESGA EHWNTVDPFYHKLAELLRESGA EHWNTVDPFYHKLAELLRESGA EHWNTVDPFYHKLAELLRESGA EHWNTVDPFYHKLAELLRESGA EHWNTVDPFYHKLAELLRESGA EHWNTVDPFYHKLAELLRESGA EHWNTVDPFYHKLQELLRESGA EHWNTVDPFYHRLGELRESGA EHWNTVDPFYHRLGELLRESGA EHWNTVDPFYHRLGELLRESGA EHWNTVDPFYHLRESGA EHWNTVDPFYHRLGELLRESGA EHWNTVDPFYHLRESGA EHWNTVDPFYHRLGELLRESGA II.0 EHWNTVDPFYHRLGELLRESGA II.0 II.1 EHWNTVDPFYHLRESGA II.0 II.1 II.1 EHWNTVDPFYHLRESGA II.0 II.1 II.1 III.1 II	B6C-3-C10-IGFR	EHWNTVDPFYNKLSELLRESGA	30.0	17.7	i	1	1
EHWNTVDPFYARLSALLRESGA EHWNTVDPFYARLSALLRESGA EHWNTVDPFYHILAELLRESGA EHWNTVDPFYHILAELLRESGA EHWNTVDPFYHILAELLRESGA EHWNTVDPFYPURLTELLRESGA EHWNTVDPFYDRLTELLRESGA EHWNTVDPFYHKLSELLRESGA EHWNTVDPFYHKLSELLRESGA EHWNTVDPFYHKLAELLRESGA EHWNTVDPFYHKLAELLRESGA EHWNTVDPFYHKLAELLRESGA EHWNTVDPFYHKLAELRESGA EHWNTVDPFYHKLAELRESGA EHWNTVDPFYHKLAELRESGA EHWNTVDPFYHKLAELRESGA EHWNTVDPFYHKLAELLRESGA EHWNTVDPFYHKLAELRESGA EHWNTVDFYHKLAELRESGA EHWNTVDFYHKLAELLRESGA EHWNTVDFYHKLAELRESGA B6C-3-F7-IGFR	EHWNTVDPFYQKLSELLRESSA	27.3	17.6	1	1	1	
EHWNTVDPFYARLSALLRESGA EHWNTVDPFYHILAELLRESGA EHWNTVDPFYHILAELLRESGA EHWNTVDPFYHKLGGRLRESGA EHWNTVDPFYDRLTELLRESGA EHWNTVDPFYPKLELLRESGA EHWNTVDPFYPKLSELLRESGA EHWNTVDPFYHKLSELLRESGA EHWNTVDPFYHKLAELLRESGA EHWNTVDPFYHKLAELLRESGA EHWNTVDPFYHKLAELLRESGA EHWNTVDPFYHKLAELLRESGA EHWNTVDPFYHKLAELLRESGA EHWNTVDPFYHKLAELLRESGA EHWNTVDPFYHKLQELLRESGA EHWNTVDPFYHKLQELLRESGA EHWNTVDPFYHKLGELLRESGA EHWNTVDPFYHKLGELRESGA EHWNTVDPFYHKLGELRESGA EHWNTVDFYHKLGELRESGA EHWNTVDFYHKLGELRESGA EHWNTVDFYHKLGELRESGA EHWNTVDFYHKLGELRESGA EHWNTVDFYHKLGELRESGA EHWNTVGAFQPTPEXLLRESGA EHWNTVGAFQPTPEXLLRESGA EHWNTVGAFQPTPEXLLRESGA EHWNTVGAFQPTPEXLLRESGA EHWNTVGAFQPTPEXLLRESGA EHWNTVGAFQPTPEXLLRESGA EHWNTVGAFQPTPEXLLRESGA	B6C-3-F10-IGFR	EHWNTVDPFYAKLSALLRESGA	27.4	17.4	1	1	1
EHWNTVDPFYHILAELLRESGA EHWNTVDPFYHKLCGRLRESGA EHWNTVDPFYDRLTELLRESGA EHWNTVDPFYDRLTELLRESGA EHWNTVDPFYPRESGA EHWNTVDPFYHKLSELLRESGA EHWNTVDPFYHKLSELLRESGA EHWNTVDPFYHKLAELLRESGA EHWNTVDPFYHKLAELLRESGA EHWNTVDPFYHKLAELLRESGA EHWNTVDPFYHKLAELLRESGA EHWNTVDPFYHKLAELLRESGA EHWNTVDPFYHKLQELLRESGA EHWNTVDPFYHKLQELLRESGA EHWNTVDPFYHKLQELLRESGA EHWNTVDPFYHKLGELLRESGA EHWNTVDPFYHKLGELLRESGA EHWNTVDPFYHKLGELLRESGA EHWNTVDPFYHKLGELLRESGA EHWNTVDPFYHKLGELLRESGA EHWNTVDPFYHKLGELLRESGA EHWNTVDFYHKLGELLRESGA EHWNTVDFYHKLGELLRESGA EHWNTVDFYHKLGELLRESGA EHWNTVDFYHKLGELLRESGA EHWNTVGAFQPTPEXLLRESGA EHWNTVGAFQPTPEXLLRESGA EHWNTVGAFQPTPEXLLRESGA	B6C-3-C12-IGFR	EHWNTVDPFYARLSALLRESGA	28.4	17.3	1	1	i i
EHWNTVDF*YHKLCGRLRESGA EHWNTVDPFYDRLTELLRESGA EHWNTVDPFYPRLTELLRESGA EHWNTVDPFYPRLLRESGA EHWNTVDPFYHKLSELLRESGA EHWNTVDPFYHKLSELLRESGA EHWNTVDPFYHKLAELLRESGA EHWNTVDPFYHKLAELLRESGA EHWNTVDPFYHKLAELLRESGA EHWNTVDPFYHKLAELLRESGA EHWNTVDPFYHKLQELLRESGA EHWNTVDPFYHKLQELLRESGA EHWNTVDPFYHKLQELLRESGA EHWNTVDPFYHKLQELLRESGA EHWNTVDPFYHKLQELLRESGA EHWNTVDFYHKLQELLRESGA EHWNTVDFYHKLGELLRESGA EHWNTVDFYHKLGELLRESGA EHWNTVDFYHKLGELLRESGA EHWNTVGAFQPTPEXLLRESGA EHWNTVGAFQPTPEXLLRESGA EHWNTVGAFQPTPEXLLRESGA EHWNTVGAFQPTPEXLLRESGA EHWNTVGAFQPTPEXLLRESGA EHWNTVGAFQPTPEXLLRESGA EHWNTVGAFQPTP	B6C-3-E11-IGFR	EHWNTVDPFYHILAELLRESGA	28.0	17.2	1	i i	1
EHWNTVDPFYDRLTELLRESGA EHWNTVDPF*PKVSELLRESGA EHWNTVDPFYHKLSELLRESGA EHWNTVDPFYHKLSELLRESGA EHWNTVDPFYHKLSELLRESGA EHWNTVDPFYHKLAELLRESGA EHWNTVDPFYHKLAELLRESGA EHWNTVDPFYHKLAELLRESGA EHWNTVDPFYHKLAELLRESGA EHWNTVDPFYHKLAELLRESGA EHWNTVDPFYHKLGELLRESGA EHWNTVDPFYHKLGELLRESGA Z.3 11. R EHWNTVDPFLDKRESGA Z.3 10. R EHWNTVDPFLDKRESGA EHWNTVDPFLLRESGA Z.3 10.	B6C-3-F8-IGFR	EHWNTVEP*YHKLCGRLRESGA	25.4	16.0	1	! !	i
EHWNTVDPF*PKVSELLRESGA EHWNTVDPFYHKLSELRESGA EHWNTVDPFYHKLSELLRESGA EHWNTVDPFYHKLSELLRESGA EHWNTVDPFYHKLAELLRESGA EHWNTVDPFYHKLAELLRESGA EHWNTVDPFYHKLAELLRESGA EHWNTVDPFYHKLAELLRESGA EHWNTVDPFYHKLAELLRESGA EHWNTVDPFYHKLAELLRESGA EHWNTVDPFYHKLGELLRESGA EHWNTVDPFYHKLGELLRESGA 2.3 11. R EHWNTVDPFLDKRESGA 2.3 10. EHWNTVDPFLDKRESGA 2.3 9.	B6C-3-F2-IGFR	EHWNTVDPFYDRLTELLRESGA	30.8	15.1	I I	1	1
EHWNTVEPFGA*LAEPLRESGA ERWNTVDPFYHKLSELLRESGA EHWNTVDQFYQALFELLRESGA EHWNTVDQFYQALFELLRESGA EHWNTVDPFYHKLAELLRESGA EHWNTVDPFYHKLAELLRESGA EHWNTVDPFYHKLAELLRESGA EHWNTVDPFYHKLAELLRESGA EHWNTVDPFYHKLQELLRESGA 2.3 11. R EHWNTVDFFLRESGA 2.3 10. R EHWNTVDFFLDKRS * LLRESGA 2.7 10. R EHWNTVDFLLRESGA EHWNTVDFLLRESGA 2.7 10.	B6C-3-B6-IGFR	EHWNTVDPF*PKVSELLRESGA	•	14.7	!	1	1
ERWNTVDPFYHKLSELLRESGA EHWNTVDQFYQALFELLRESGA EHWNTVDPFYHKLAELLRESGA EHWNTVDPFYHKLAELLRESGA EHWNTVDPFYHKLAELLRESGA EHWNTVDPFYHKLAELLRESGA ETWNTVDPFYHKLAELLRESGA 2.3 11. R EHWNTVDPFYHKLQELLRESGA 2.3 11. R EHWNTVDPFLDKRS*LLRESGA 2.7 10. R EHWNTVDPFLDKRS*LLRESGA 2.7 10.	B6C-3-D5-IGFR	EHWNTVEPFGA*LAEPLRESGA	2.4	14.3	1	i I	1
EHWNTVDQFYQALFELLRESGA EHWNTVDPFYHKLAELLRESGA EHWNTVDPFYHKLAELLRESGA EHWNTVDPFYHKLAELLRESGA EYWNTVDPFYHKLAELLRESGA EYWNTVDPFYHKLAELLRESGA EHWNTVDPFYHKLQELLRESGA 2.3 11. R EHWNTVDPFYHKLQELLRESGA 23.3 10. R EHWNTVDPFLDKRS*LLRESGA EHWNTVOAFQPTPEYLLRESGA EHWNTVOAFQPTPEYLLRESGA 2.7 10.	B6C-3-A4-IGFR	ERWNTVDPFYHKLSELLRESGA	2	•	1	I 	! !
EHWNTVDPFYHKLAELLRESGA EHWNTVDPFYDKLSDLKESGA EHWNTVDPFYHKLAELLRESGA EHWNTVDPFYHKLAELLRESGA EHWNTVDPFYHKLQELLRESGA EHWNTVDPFYHKLQELLRESGA EHWNTVDPFYHKLQELLRESGA 2.7 10. R EHWNTVDPFLDKRS*LLRESGA EHWNTVDPFLDKRS*LLRESGA EHWNTVDPFLDKRS*LLRESGA EHWNTVGAFQPTPEYLLRESGA 2.7 10.	B6C-3-D3-IGFR	EHWNTVDQFYQALFELLRESGA		13.8	1	!	\$ 1
EHWNTVDPFYDKLSDLLRESGA EYWNTVDPFYHKLAELLRESGA EHWNTVRPRYQ*LSELLRESGA EHWNTVDPFYHKLQELLRESGA EHWNTVDPFLDKRSGA 2.3 11. R EHWNTVDPFLDKRSGA EHWNTVDPFLDKRS*LLRESGA EHWNTVDPFLDKRS*LLRESGA EHWNTVGAFQPTPEYLLRESGA 2.3 9.	B6C-3-F9-IGFR	EHWNTVDPFYHKLAELLRESGA	4.	13.6	1	:	1 1
R EYWNTVDPFYHKLAELLRESGA 17.1 11. R EHWNTVRPRYQ*LSELLRESGA 2.3 11. R EHWNTVDPFYHKLQELLRESGA 23.3 10. R EHWNTVDPFLDKRS*LLRESGA 2.7 10. R EHWNTVDPFLDKRS*LLRESGA 2.3 9.	B6C-3-A7-IGFR	EHWNTVDPFYDKLSDLLRESGA	14.4	•	t I	ł	1
EHWNTVRPRYQ*LSELLRESGA EHWNTVDPFYHKLQELLRESGA EHWNTVATF*DKVTDLLRESGA EHWNTVDPFLDKRS*LLRESGA 2.7 10. 2.7 10. EHWNTVDPFLDKRS*LLRESGA 2.3 9.	B6C-3-H10-IGFR	EYWNTVDPFYHKLAELLRESGA	17.1	11.1	!	1	1
EHWNTVDPFYHKLQELLRESGA EHWNTVATF*DKVTDLLRESGA 2.7 10. EHWNTVDPFLDKRS*LLRESGA 2.3 9. EHWNTVGAFQPTPEYLLRESGA	B6C-3-H11-IGFR	EHWNTVRPRYQ*LSELLRESGA	2.3	11.0	1	1	i i
EHWNTVATF*DKRTDLLRESGA 2.7 10. EHWNTVDPFLDKRS*LLRESGA 2.3 9. EHWNTVGAFQPTPEYLLRESGA	B6C-3-A1E-IGFR	EHWNTVDPFYHKLQELLRESGA	Э.	•	t t	1	1
EHWNTVDPFLDKRS*LLRESGA 2.3 9. EHWNTVGAFQPTPEYLLRESGA	B6C-3-E12-IGFR	EHWNTVATF*DKVTDLLRESGA	2.7	10.1	1	1 1	1
	B6C-3-H12-IGFR	EHWNTVDPFLDKRS*LLRESGA	2.3	•	1	1	i t
	B6C-3-G1-IGFR	EHWNTVGAFQPTPEYLLRESGA	1 t	:	1	1	1

			Ratios ove	Ratios over Background	•	Comparisons	sons IR/IGFR
Clone	Sequence		E-Tag	IGFSK :-	:		
Design	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX		30.6	15.1	4.2	3.6	0.3
Parental	FYDAIDQLVRGSARAGGTRD		19.9	1.2	13.9	0.1	11.5
20E2A-4-F9-IR			•	1.3	7.6	0.1	٠.
20E2A-4-E2-IR	IGRVRSFYDALDKLFQSDWER)		1.6	6.1	0.3	•
20E2A-3-B6-IR	RDAGSSFYDAIDQLVCL11FC		18.6	4.2	15.2	0.3	9. M
20E2A-3-A7-IR	MPMGLNFYDG1EQLVREWGGD		20.1	10.6	15.8	0.7	1.5
20E2A-4-F7-IR	TISAHTFYEALYQLIEGIUFU		24.4	16.4	23.8	0.7	1.4
20E2A-3-C9-IR	SPWGRAFYDALDQLMGGAEKG			5.9	9.9	0.9	1.1
20E2A-3-C11-IR	LSPPRDFYDAIQQLVRDGGWG		17.2	8.3	8.9	6.0	•
20E2A-4-G7-IR	HGVPRTFYDAIDQLVWG1EVG		25.4	26.9	25.6	1.1	1.0
20E2A-4-H11-IR	GGTDQLFYGAIDQLVGGTWWK		. 4	2.1	2.1	1.0	1.0
20E2A-4-E9-IR	LSVHQSFYDAINELLFSGLEA		12.6	5.6	5.7	1.0	1.0
20E2A-4-F4-IR	GDARDPFY DAME OLV I GELGG		21.0	11.9	12.1	1.0	1.0
20E2A-3-A3-IR	VASPRSFYEALAQLVFNLGQL			16.0	16.1	1.0	1.0
20E2A-4-E4-IR	RKPCQ'IFYDCI LDLV V 1DVDV		22.1	19.5	18.8	1.0	1.0
20E2A-4-G5-IR	LLSKWTYYDALEQLVGGGADG			20.4	21.2	1.0	1.0
20E2A-4-H1-IR	PAGCOGETERALECENT TO TOTAL TO		22.3	20.8	20.3	1.0	1.0
20E2A-3-D8-IR	AVFFRIFIEALDQDVGVSDDG			21.0	20.4	1.0	1.0
20E2A-4-F11-1K				22.3	22.5	1.0	1.0
20E2A-3-C1-IR	OCNERT FIBRIAGN 16CD VS		24.6	22.5	22.8	1.0	1.0
20E2A-3-B2-IR				22.9	22.2	1.0	1.0
20E2A-4-G10-IR	HHSAFSFIDALAQUVGVFWED		24.6	23.4	22.6	1.0	1.0
20E2A-3-A5-IR	F.T.YVHSFYDALEQDVRGEGGG		4		25.1	1.0	1.0
20E2A-4-H3-1R	CONACINE IDAILACHUCE COLOGO		25.2	24.4	24.7	1.0	1.0
20E2A-3-C4-1R	SOLDWIF IDALDXDVOKEKON		18.5	15.7	13.6	1.2	o.0
20E2A-4-E3-1K	PUGCAIFINAIKENVIGIICV PODDMTEVDAIAOINAOSADG		17.8	16.6	14.4	1.2	6.0
20E2A-4-E7-1K	RGFFILL LDALAXIAVINA						

į		Ratios ove	Ratios over Background	~	Comparisons	isons IR/IGFR
Clone	Sequence xxxxxxevbaTDOLVXXXXXX	9	;	:	; ;	;
20E28-3-B3-TB	PSSCOS FYDAT FRI VI GGTCG	22.4	18.6	15.9	1.2	6.0
-H10-	VSRKFSFYDAIOOLVRGDAGV	24.8	24.5	21.2	1.2	6.0
- 52 - 58 18		4.3	2.2	2.1	1.1	6.0
- H5	RGSATTFYDAINOLVGODGGW	21.3	18.3	16.5	1.1	6.0
: ב	AOPCVSFYDAIEOLVTGRSCM	21.4	18.3	16.0	1.1	6.0
- 52	GGDGDPFYDWIEOLVRAGSEA	20.1	20.5	18.2	1.1	6.0
- H7		22.6	21.2	18.6	1.1	6.0
20E2A-3-D5-IR		22.7	21.3	18.9	1.1	•
20E2A-3-A1-IR	EVNALSFYDAIDOLVRGGLGG	23.8	21.7	19.9	1.1	6.0
20E2A-4-H9-TR	RIOPRIFYEAIDOLIGGVLEG	24.0	22.5	20.8	1.1	6.0
-177	SGAHRTFYDAIOELVGMGGSK	24.1	23.5	21.0	1.1	6.0
-4-E5		24.1	23.5	20.9	1.1	6.0
E E		22.5	14.6	11.7	1.3	0.8
20E2A-3-A4-IR	SOCRGGFYDAIYQLVTGVNCI	20.2	17.5	13.4	1.3	0.8
20E2A-4-G8-IR	DRLAFSFYDAIDQLVHCCGHG	21.7	18.0	13.8	1.3	0.8
20E2A-3-B7-IR		21.1	21.4	16.9	1.3	0.8
20F2A-4-G11-IR	GGSVLSFYDAIAQLVGGGQSI	22.9	23.1	17.7	1.3	0.8
20E2A-3-C5-TB	RSGPMSFYDAIEOLVLGRLHP	24.2	24.3	19.0	1.3	0.8
20E2A-4-E8-IR	VSGCRTFYDAIDQLVSGQACG	17.1	11.5	9.4	1.2	0.8
20E22-4-H2-TR	AOFPRIFYDAIEOLIHGKGMD	21.6	13.7	11.6	1.2	0.8
20E2A-4-H4-TR	CAOPESFYDAIDRLVTGRCLV	21.3	19.6	16.3	1.2	•
20E2A-3-D4-IR	PDECOSFYCAIDRLVTGKGGR	23.2	22.2	18.0	1.2	0.8
20E2A-4-F5-IR	ORRARDFYEAIQQLVGGVAGL	12.2	5.7	3.8	1.5	0.7
20E2A-3-B10-TR	PLVRGTFYDAIKOLVMGGSSD	14.9	5.9	3.9	1.5	0.7
20E21. 3 E40 IR	VGIAWTFYDAIOOLVRGSPEG	15.5	11.0	7.2	1.5	0.7
20E2A-3-D10-TR	PRGOASFYDMIEOLVGSADWN	22.2	19.1	12.8	1.5	0.7
20E2: 3 DZ2 EX	DGRVWSFYDALEOLVGOFEGP	21.8	19.3	13.0	1.5	0.7
OESA	RFVVRSFYDAIEOLILAPNLG	21.3	19.9	13.3	1.5	0.7
20E2A-4-E1-TR	KVGRGSFYDAIRELVGOGGHV	23.1	20.7	13.6	1.5	0.7
20E2A-4-F12-TR	PAIGFTFYDAIROLVWFOGAD	17.5	17.1	12.1	1.4	0.7
20E2A-4-G3-IR	ALPGRSFYDAIAQLVGPDWGA	21.6	19.4	14.1	1.4	0.7
	1					

FIGURE 2L (Con't)

		D.			- :	
	Sequence	E-Tag	IGFSR	R	IGFR/IR	IR/IGFR
	XXXXXXFYDAIDQLVXXXXXX	\$ \$	1	;	t I	1 1
20E2A-3-C2-IR	RPOGGTFYDMIKQLVLGSGWG	23.4	20.9	15.4	1.4	0.7
20E2A-3-B1-IR	WSAFADFYDAIQHLVAGEVGA	22.1	21.6	15.6	1.4	0.7
20E2A-3-A8-IR	SDGRDGFYDAIÖQLVRSAFGD	12.3	4.8	2.7	1.8	9.0
20E2A-4-G2-IR	IRSVFSFYDAIDQLVGKGGWS	18.9	13.8	7.9	1.8	9.0
20E2A-3-A9-IR	GGVSLTFYEAIEQLVRGGFDA	23.3	20.3	11.3	1.8	9.0
20E2A-3-D3-IR	AAOAFSFYDLINOLVASKPSE	24.4	24.5	13.5	1.8	9.0
20E2: 3 E3 E3	OSGACGEYDAINOLVLGVSIC	13.5	4.6	2.7	1.7	9.0
20E211 3 111 111	GGIVESFYEAIDOLVRGNGAG	21.4	15.3	8.9	1.7	9.0
-:: TR	IYTGOGFYDAIEOLVRGGSTP	22.3	19.0	11.3	1.7	9.0
TR	KSPALSFYDAIEOLVGSOGVR	22.5	19.0	11.2	1.7	9.0
IR	ISPPWTFYDAIDOLVGGSDGR	14.5	6.2	3.9	1.6	9.0
20E2A-3-D1-IR	GSRFRGFYDAIDÖLVRQGGLE	16.5	9.9	4.0	1.6	9.0
-IR	GVAGGTFYDAIEQLVRÖFGGS	20.2	14.4	8.9	1.6	9.0
IR	RPLRWSFYDALDQLVGSAIGG	23.9	22.5	14.2	1.6	9.0
20E2A-3-C12-IR	MOGRGGFYDAIADLVGGHVRG	21.3	23.0	14.4	1.6	9.0
20E2A-3-A2-IR	TSOGLSFYDAINQLVAGGWGG	18.9	11.6	7.5	1.5	9.0
20E2A-3-C7-IR	$\operatorname{SGGTVTFYDAINOLVOGRYNG}$	21.6	15.1	6.9	2.2	0.5
20E2A-3-C10-IR	GGALDPFYDAIYOLVIRGSSG	18.1	18.0	9.1	2.0	0.5
20E2A-3-D9-IR	KORGVTFYDLLNÖLVGGSARG	21.8	21.6	8.4	2.6	0.4
- IR	PRAPRSFYDAIHÕLVGRQGPG	24.3	18.1	7.4	2.5	0.4
20E2A-3-A6-IR	PCSDDOFYDALSQLVGIRVCP	17.8	19.1	7.6	2.5	0.4
20E2A-4-G12-IR	SYGYOSFYDAIEELVRGPPAR	0.6	9.3	2.6	3.6	0.3

		Ratios ove	Ratios over Background	pun	Comparisons	risons
Clone	Sequence	E-Tag	IGFSR	¥	IGFR/IR	IR/IGFR
Design	XXXXXXFYDAIDQLVXXXXXX	l I	1	:	:	!
Parental	FYDAIDQLVRGSARAGGTRD	30.6	15.1	4.2	3.6	0.3
20E2A-4-F11-IGFR	QGGSASFYDAIDRLLRMRIGG	21.3	18.8	1.3	14.6	0.1
20E2A-4-F12-IGFR	ĀQGSEGFYDALAQLVGQLVSG	23.3	23.9	3.1	7.8	0.1
20E2A-3-B4-IGFR	GHPAVSFYDAIDQLLRRRGGG	21.8	16.6	2.4	6.9	0.1
20E2A-4-F4-IGFR	YSDTYSFYDAIVQLVRRGASA	20.7	20.0	3.6	5.5	0.2
20E2A-3-C7-IGFR	VGTVAGFYDAIAQLVARASRV	17.6	5.4	1.1	5.1	. 0.2
20E2A-3-C10-IGFR	RFVWGSFYDAIDQLVQGRWRG	23.3	21.0	4.2	5.0	0.2
20E2A-3-D6-IGFR	RAVGDSFYEAIQQLVRGGHGV	15.1	11.8	2.4	5.0	0.2
20E2A-4-F6-IGFR	LRSQLSFYEAIDQLVQWKGGA	21.5	19.9	4.3	4.6	0.2
20E2A-3-A8-IGFR	DKFFTSFYDAIDQLVQSVRGV	22.2	13.3	2.9	4.6	0.2
20E2A-4-F9-IGFR	MQSGFSFYDAIDRLVGRLGER	21.2	19.0	4.4	4.4	0.2
20E2A-4-F3-IGFR	VGSSSFYEAIERLVQGLGRH	20.6	19.3	4.6	4.2	0.2
20E2A-3-B2-IGFR	LSWAAGFYEAIDQLVRSGGHR	18.7	14.7	3.8	3.9	0.3
20E2A-4-G8-IGFR	QQVHAGFYEALEELVGFGFLG	20.9	10.8	2.7	3.9	0.3
20E2A-3-D10-IGFR	MMVVDGFYDALHQLVVAQSLG	20.6	6.9	1.8	3.9	0.3
20E2A-3-A12-IGFR	LSVALSFYDALGQLVAGEGRW	16.1	4.3	1.1	3.9	0.3
20E2A-3-A11-IGFR	SGSNLGFYDALRQLVGATDGS	17.8	9.7	2.6	3.7	0.3
20E2A-4-H1-IGFR	PSGFLSFYEAIDQLVHGVRWF	20.8	14.5	4.1	3.5	0.3
20E2A-4-F7-IGFR	AFTPTSFYDAIEQLVQQLSPR	19.5	17.9	5.3	3.4	0.3
20E2A-3-D7-IGFR	VSSLRSFYDALDELVRRPFQQ	22.0	18.3	5.6	3.3	0.3
20E2A-3-A9-IGFR	VSMPQSFYDALKQLVRGISEG	24.7	10.5	3.2	3.3	0.3
20E2A-3-A10-IGFR	IGVSRGFYDAIDKLVRDRGSP	26.3	15.4	4.8	3.2	0.3
20E2A-3-B11-IGFR	GRSLLSFYDLIDQLVQAGNGG	15.8	10.7	3.4	3.2	0.3
20E2A-3-D12-IGFR	GQRAQSFYEALARLVCEGRCT	13.9	9.0	2.8	3.2	0.3
20E2A-4-H11-IGFR	CRFQGSFYDAIDLLVLGVRTC	22.8	17.5	5.7	3.1	0.3
20E2A-4-H5-IGFR	RWAFQSFYDAIDHLVNHREGH	20.1	16.6	5.5	3.0	0.3
20E2A-4-E11-IGFR	LPPSSGFYNAIQQLVCGHRGC	21.0	12.6	4.2	3.0	0.3

Clone	Seunence	Ratios ov E-Tag	Ratios over Background E-Tag IGFsR II	~	Comparisons IGFR/IR IR/IG	risons IR/IGFR
Design	XXXXXXFYDAIDQLVXXXXXX		:	:		1
20E2A-4-F2-IGFR	TGVFNDFYDALQQLVGFRVRD	19.4	19.0	6.8	2.8	0.4
20E2A-3-D4-IGFR	YGSFETFYDAIDQLVRRGSQP	16.1	11.8	4.2	2.8	0.4
20E2A-3-B10-IGFR	RQLLDSFYEAIDQLVRSESRP	24.0	14.3	5.3	2.7	0.4
20E2A-4-E5-IGFR	WPRGDPFYDAMEKLLSQGGGR	18.1	20.6	7.9	2.6	0.4
20E2A-3-D5-IGFR	PGLIQSFYDAIDQLVRQGRGN	15.1	9.3	3.6	2.6	0.4
20E2A-4-G11-IGFR	MNVFVSFYDAIDQLVCQRIGC	20.7	3.3	1.3	2.6	0.4
20E2A-3-C12-IGFR	LDMIGGFYEAIDQLVSGSLAP	25.9	17.4	7.2	2.4	0.4
20E2A-4-G5-IGFR	RRPCNSFYDAIQQLLVGGPCG	23.6	14.6	6.0	2.4	0.4
20E2A-3-D9-IGFR	FGRRSTFYDLIDQLVGQGRGT	19.8	12.3	5.1	2.4	0.4
20E2A-4-F10-IGFR	LRAPRSFYEAIYQLAQRPSVP	21.4	21.6	9.3	2.3	0.4
20E2A-4-E2-IGFR	VQRFSSFYDALDQLVGHGVWK	22.6	21.3	9.1	2.3	0.4
20E2A-4-E3-IGFR	PSARMGFYDLIDQLVGLVPGS	21.0	21.8	10.1	2.2	0.5
20E2A-3-C4-IGFR	SLQPHDFYDAIHRLVFHGGRF	23.5	17.4	7.8	2.2	0.4
20E2A-3-C5-IGFR	ERHGGSFYDAIAQLLQSDRSR	22.2	17.1	7.7	2.2	0.4
20E2A-4-G12-IGFR	YQPPGSFYDWIRELVAGPRRE	24.3	16.3	7.4	2.2	0.5
20E2A-3-C3-IGFR	FAHASSFYDAIDQLVAKCQSP	11.3	2.7	1.2	2.2	0.5
20E2A-3-B7-IGFR	AQSSSGFYEALYQLVWGRGPG	22.3	22.6	10.8	2.1	0.5
20E2A-4-E10-IGFR	TTSGGSFYDAMYQLVWGDWRR	22.6	19.9	9.4	2.1	0.5
20E2A-4-E6-IGFR	ARGTAGFYAELERLVRGQDHG	23.0	16.6	7.9	2.1	0.5
20E2A-3-D3-IGFR	PRHAINFYDAIHQLVFGPGRQ	20.5	15.7	7.6	2.1	0.5
20E2A-4-G4-IGFR	QSAHWSFYDA I ERLVNMDTMP	22.6	14.5	7.0	2.1	0.5
20E2A-4-F1-IGFR	VGVVSSFYDAIDQLVGWDRGS	19.6	19.9	9.8	2.0	0.5
20E2A-4-H7-IGFR	DTLIASFYDAIDQLVRLGRNQ	23.0	17.1	8.7	2.0	0.5
20E2A-3-B9-IGFR	FQGTQGFYDAIERLMRRGERP	26.4	22.1	11.5	1.9	0.5
20E2A-4-E9-IGFR	WADWGSFYDAIEQLVQRGGGV	25.3	20.7	11.1	1.9	0.5
20E2A-3-B1-IGFR	EQLSCGFYDAIHQLVHGGGLG	23.1	17.9	9.5	1.9	0.5
20E2A-4-G1-IGFR	CGGRCSFYDAIDQLVGWLPGA	22.6	17.7	9.3	1.9	0.5
20E2A-4-E7-IGFR	MMRVDGFYEAIDRLVNEGQAT	17.2	8.6	4.6	1.9	0.5
20E2A-3-D11-IGFR	RGQATSFYEAIDQLMGGSGGV	16.1	6.1	3.2	1.9	0.5
20E2A-3-A3-IGFR	GHYFGSFYDAIDQLVAGMLPG	5.2	3.0	1.5	1.9	0.5
20E2A-4-G7-IGFR	PEGVQGFYDALAHLVGGSLFG	24.4	21.1	11.5	1.8	0.5

FIGURE 2M (Con't)

		Ratios ov	Ratios over Background	pun	Comparisons	suo
Clone	Sequence	E-Tag	IGFsR	=	IGFR/IR II	IR/IGFR
Design	XXXXXXFYDAIDQLVXXXXXX	1	1	:	1 1	1
20E2A-4-G2-IGFR	IGVLGSFYDAIDQLVRQGGNR	22.3	17.5	9.9	1.8	9.0
20E2A-4-G3-IGFR	RDVADGFYAAIEQLVRGQFGL	21.2	12.3	6.9	1.8	9.0
20E2A-3-B5-IGFR	VRQAKSFYDAIDQLVRGĀLRG	24.0	22.7	13.3	1.7	9.0
20E2A-4-H4-IGFR	QVFRGSFYDAIDALVRWGGRA	22.2	20.6	12.0	1.7	9.0
20E2A-4-F8-IGFR	VGAAFSFYDAIDQLVGWSPGS	17.3	17.9	10.7	1.7	9.0
20E2A-3-A6-IGFR	PSPVWSFYDAIQQLVRSGQRG	23.8	23.7	15.0	1.6	9.0
20E2A-4-H12-IGFR	PVSATSFYDAINQLVRMGSRG	25.1	23.5	14.2	1.6	9.0
20E2A-3-B12-IGFR	VMRRDRFYDAIEQLVGGRIGV	27.6	21.9	13.6	1.6	9.0
20E2A-3-B8-IGFR	TTYVNSFYDALQQLLGGDADV	21.5	19.0	12.2	1.6	9.0
20E2A-3-C8-IGFR	LSNMITFYDAINQLVGHVQSL	23.2	17.7	11.4	1.6	9.0
20E2A-4-H10-IGFR	ASSRLSFYDAIEQLIKWSPGP	25.3	23.8	16.2	1.5	0.7
20E2A-3-C9-IGFR	WDLVDSFYDAIDQLVGQRVPG	25.4	21.8	14.6	1.5	0.7
20E2A-4-H2-IGFR	FAFVGSFYDALAQLVAQGPRS	21.8	20.1	13.0	1.5	9.0
20E2A-3-B6-IGFR	EDQPNSFYDAIRQLVMGRLSP	20.3	18.1	11.8	1.5	0.7
20E2A-4-G9-IGFR	SVGPRSFYDAIDQLVGGAWVG	26.0	16.1	10.8	1.5	0.7
20E2A-4-H6-IGFR	KFRVYTFYDAIDQLVNQGRGR	21.9	19.6	13.9	1.4	0.7
20E2A-4-H9-IGFR	GRGWGSFYEAIDQLVRGLGET	24.9	16.8	11.8	1.4	0.7
20E2A-4-G10-IGFR	FTSFHTFYDAIEQLVGQGGDP	25.3	16.5	12.1	1.4	0.7
20E2A-3-A4-IGFR	AGSVTSFYDAMEQLVATGTSA	16.8	2.5	1.8	1.4	0.7
20E2A-3-A7-IGFR	PRESFSFYDAIHQLVTGRVRS	26.0	24.9	19.3	1.3	0.8
20E2A-4-E12-IGFR	LGRADGFYDAIKQLVGADWGG	23.3	23.1	17.8	1.3	0.8
20E2A-3-D1-IGFR	RSGTWTFYDALELLVQGSGSR	24.0	22.4	17.6	1.3	0.8
20E2A-3-C6-IGFR	PVVLFSFYDAIDQLVRKGLGP	23.7	21.7	17.2	1.3	0.8
20E2A-3-D2-IGFR	GRRAQTFYDALEQLVGGEALG	21.4	15.1	11.4	1.3	0.8
20E2A-4-E8-IGFR	AGPDMSFYDAIDQLVHCCGPF	18.4	13.6	10.4	1.3	0.8
20E2A-4-G6-IGFR	HGEKLSFYDAIAQLVGFDIGH	24.7	21.9	17.7	1.2	0.8
20E2A-4-F5-IGFR	GYTPVDFYDAIRQLVTGGWPG	21.7	21.7	18.2	1.2	0.8
20E2A-3-C2-IGFR	FGGFSSFYDALDQLARGRGSD	22.5	19.6	15.8	1.2	0.8
20E2A-4-H8-IGFR	VGIVRGFYEAIERLVGDTHGQ	24.4	18.5	15.1	1.2	0.8
20E2A-3-A5-IGFR	TPGGFSFYDAIQQLVDVLSDS	22.7	15.6	12.6	1.2	0.8
20E2A-3-C11-IGFR	TNAALTFYDAIEQLVRWG <u>o</u> rd	25.8	24.3	21.2	1.1	6.0

FIGURE 2M (Con't)

			Ratios ov	Ratios over Background	pun	Comparisons	suos
Clone	Sequence		E-Tag	IGESR	R	IGFR/IR II	VIGFR
Design	XXXXXXFYDAIDQLVXXXXXX		1	1	!	:	1
20E2A-3-C1-IGFR	GOSPLSFYDAIDQLVRAFPVG		23.4	22.4	20.5	23.4 22.4 20.5 1.1 0.9	6.0
20E2A-3-B3-IGFR	AĞQLGGFYIAICĞLVGYEYCT		21.0	17.0	14.8	1.1	6.0
20E2A-3-D8-IGFR	SAGPLSFYDAIAQLVGPAWRL	1	22.0	22.0 19.7 19.6	19.6	1.0 1.0	1.0

ē	,	Ratios ove	Ratios over Background	pun	Comparisons	isons
Cione Design	Sequence XXXXXXFYXXhXXhXXXXX	E-Tag	IGFsR 	≅ :	IGFR/IR	IR/IGFR
Parental	FYDAIDQLVRGSARAGGTRD	30.6	15.1	4.2	3.6	0.3
20E2Bα-3-B3-IR	AGVNAGFYRYFSTLLDWWDQG	33.5	1.2	23.5	0.1	20.0
20E2Bα-4-F12-IR	SVKEVQFYRYFYDLLQSEESG	35.5	5.9	27.8	0.2	4.7
20E2Ba-3-B8-IR	IEVTQPFYDYFQQLLRLYGND	39.3	18.2	36.5	0.5	2.0
20E2Ba-3-D2-IR	VQCRADFYSYFACLVGRPGSR	42.6	19.7	26.7	0.7	1.4
20E2Bα-3-A5-IR	RNYPIGFYQFFHELVISSGGG	36.9	22.7	24.5	0.9	1.1
20E2Ba-3-A3-IR	DLGGNSFYYGLLRLVLQDAVG	39.9	33.5	35.5	0.9	1.1
20E2Ba-4-E9-IR	CKDQPDFYMGIKCLISGGGSV	32.8	29.6	28.6	1.0	1.0
20E2Ba-4-G8-IR	ACEGGSFYGCLQSLMSVESGN	37.5	30.5	30.9	1.0	1.0
20E2Ba-4-F9-IR	AVHEDGFYDMLRKLLSEGDSS	35.6	32.5	31.1	1.0	1.0
20E2Bα-4-E7-IR	LARNDEFYRYFEQLVFGDTEG	36.0	31.6	31.2	1.0	1.0
20E2Ba-3-D5-IR	ATCASSFYAQLNCLLSDFDVM	39.5	33.1	31.8	1.0	1.0
20E2Bα-4-F7-IR	VQACQNFYDCLNTLLLLDLGG	36.6	32.9	32.5	1.0	1.0
20E2Ba-3-B12-IR	IRGADQFYQFFRELLEGSVGE	37.0	33.4	33.5	1.0	1.0
20E2Bα-3-A11-IR	RAGSRGFYEFFENLLRVGAGG	36.9	34.9	34.2	1.0	1.0
20E2Bα-3-B7-IR	AQRCADFYACIEELLAPGSWR	40.4	37.1	36.3	1.0	1.0
20E2Bα-3-B5-IR	PGGGEGFYQGLQRLILGADGG	41.6	36.4	34.5	1.1	1.0
20E2Bα-4-G1-IR	QKRSEAFYDWIADLLGQETSG	38.5	28.9	26.5	1.1	6.0
20E2Bα-4-G11-IR	WGLRDDFYRGIRCLVQWSEGC	33.2	30.1	27.8	1.1	0.9
20E2Bα-4-E10-IR	DSTVCGFYCRLAQLVAEGGSP	35.4	30.5	28.0	1.1	0.9
20E2Bα-4-F11-IR	QHSCRTFYDCIRVLMDDGQLG	32.5	29.5	28.0	1.1	0.9
20E2Ba-4-H11-IR	WSGNVDFYYMIRQLCGDVCGS	34.8	32.0	28.7	1.1	0.9
20E2Bα-4-H3-IR	QTVHRDFYAALQDLLINDLGF	38.7	34.9	30.5	1.1	0.9
20E2Bα-4-H7-IR	SSGCQDFYSCMIQLVTGGGGD	35.3	32.5	30.5	1.1	6.0

		Ratios ove	Ratios over Background	pur	Comparisons	isons
Clone	Sequence	E-Tag	IGFsR	≃ :	IGFR/IR	IR/IGFR
Design	AAAAAAFIAAIIIAAAAAA	l r	: :	1		1
20E2Bα-3-B6-IR	SGPMVGFYRGLFSLLSPEDLQ	39.7	34.9	31.5	1.1	6.0
20E2Bu-3-D1-IR	LAEPDSFYNWIAQLLEEGFAG	41.6	35.1	31.7	1.1	6.0
20E2Bα-3-A9-IR	FSGCDNFYSCIQSLWLGPGGV	37.3	35.1	32.4	1.1	6.0
20E2Bα-3-C4-IR	QVFCDNFYHCIETLLGVGQTP	39.6	36.3	33.4	1.1	6.0
20E2Bu-4-F3-IR	RGRDNQFYHGLWALLLGSGLE	37.5	36.6	33.6	1.1	6.0
20E2Ba-4-F4-IR	VSGRGGFYDAIRDLIGPRDQG	37.2	36.9	33.7	1.1	6.0
20E2Bα-3-D4-IR	PVVLDDFYVALCQLMVQGDCF	42.1	38.0	34.5	1.1	6.0
20E2Bα-4-E4-IR	PDIADPFYAFFQGLLRADTPI	40.6	38.4	35.5	1.1	6.0
20E2Bα-4-G10-IR	VAQCTDFYACIRSLVRSGSPG	32.9	31.3	27.1	1.2	6.0
20E2Bα-3-D11-IR	CSQLVSFYLGMDCLLGRGGTQ	34.0	32.5	27.9	1.2	6.0
20E2Ba-3-C8-IR	PLACADFYQCLSDLIRGGPAW	39.2	33.0	28.2	1.2	6.0
20E2Bu-4-F2-IR	VVICTGFYDCIYQLVGSHEEM	38.7	37.6	32.3	1.2	6.0
20E2Ba-4-H12-IR	CVDRRTFYEGLQCLLGGATGD	32.3	30.4	25.8	1.2	8.0
20E2Bα-4-E1-IR	VNLRDPFYQWIEALMDSAGGE	39.2	40.2	32.3	1.2	8.0
20E2Ba-4-H8-IR	LTSSTSFYDALFCLAGLQLCG	37.6	34.8	27.0	1.3	0.8
20E2Bα-3-B4-IR	DFDSSPFYRGLRQLLESRSFP	39.9	34.9	25.9	1.3	0.7
20E2Ba-4-E2-IR	HEAGWTFYDAIQCLVGGWCSK	38.8	36.3	23.5	1.5	9.0
20E2Bα-4-H1-IR	CQQWRSFYHAVSCLLGPDDPD	40.8	33.6	20.2	1.7	9.0
20E2Bα-3-A10-IR	MVDRDPFYQGLRDLIGRQEKG	32.8	32.6	18.5	1.8	9.0
20E2Bα-3-D3-IR	LGRRGGFYRGLQDLIGTQWPR	41.9	29.5	5.6	5.3	0.2

		Ratios ov	Ratios over Background	pun	Comparisons	isons
Clone	Sequence	E-Tag	IGFsR	- 4	IGFR/IR IR/IGFR	IR/IGFR
Design	XXXXXXFYXXhXXhXXXXXX	1	:	;	:	1 1
20E2B }-4-F7-IR	DALNLRFYSYFQHLMEDQVTD	26.8	3.0	24.2	0.1	8.0
20E2B[3-3-E12-IR	GNSGGSFYRYFQLLLDSDGMS	17.2	1.4	5.5	0.3	4.0
20E2B[3-4-F3-IR	GDRVPGFYDWIRQLMVDPLEV	25.2	2.0	7.7	0.3	3.9
20E2B(3-4-F6-IR	SEREDPFYRWI <u>Q</u> AMVEGVSEG	25.7	3.8	11.0	0.4	2.9
20E2B()-3-D11-IR	GSVACDFYCHMWSLVEQPAGT	14.8	3.6	4.2	6.0	1.2
20E2BB-3-E5-IR	VHPSAGFYKGLLALIGDSQLG	24.3	6.9	4.3	1.6	9.0
20E2Bβ-3-C9-IR	FCGGLSFYGCLQELLTWESPT	29.7	24.3	15.0	1.6	9.0
20E2Bβ-3-C7-IR	QSGSGDFYDWLSRLIRGNGDG	1.5	3.1	1.5	2.0	0 بر
20E2Bβ-4-H8-IR	LPRQDGFYDALRRLISEGAGG	25.8	26.9	13.2	2.0	0.5
20E2Bβ-4-G7-IR	LQPCSGFYECI ERLIGVKLSG	19.9	25.2	1.6	15.8	0.1

		Ratios o	Ratios over Background	round	Comparisons	ıs
Clone	Sequence	E-Tag	IGFR	¥	IGFR/IR I	IR/IGFR
Design	XXXXXXEXXXLXXLXXXXXXX	;	1	;	1	1
20E2B-1-A6-IGFR	GVRAMSFYDALVSVLGLGPSG	18.6	18.1	1.1	16.8	0.1
20E2B-3-C6-IGFR	VEGRGLFYDLLR <u>Q</u> LLARRQNG	17.9	16.8	1.1	14.8	0.1
20E2B-4-H3-IGFR	KLHNLMFYYGLQRLVWGAGLG	11.2	14.8	1.1	13.9	0.1
20E2B-3-C2-IGFR	GNGDGMFYQLLSLLVGRDMHV	13.1	8.9	9.0	13.8	0.1
20E2B-3-E3-IGFR	PDLHKGFYAQLAQLIRGQLLS	22.4	16.3	1.3	13.1	0.1
20E2B-4-H12-IGFR	YSCGDGFYSLLSDLLGGQFRC	6.5	9.7	0.8	12.8	0.1
20E2B-3-D2-IGFR	IQQELTFYDLLHRLVRSELGS	20.7	12.4	1.1	11.7	0.1
20E2B-3-D8-IGFR	GGTEVDFYRALERLVRGQLGL	20.4	17.7	1.6	11.3	0.1
20E2B-3-E8-IGFR	LRIANLFYQRLWDLAFGGGG	15.7	16.7	1.5	11.1	0.1
20E2B-4-F8-IGFR	PVGVQGFYEGLSRLVLGRGGW	12.3	7.3	0.8	9.7	0.1
20E2B-1-A11-IGFR	RFSTDGFYQYLLALVGGGPVG	15.0	9.5	1.0	9.7	0.1
20E2B-3-D4-IGFR	NSRDGGFYLQLERLLGFPVTG	8.1	7.9	0.8	9.6	0.1
20E2B-2-B11-IGFR	VVTPVNFYRALEALVRGQRLG	13.9	10.6	1.1	9.4	0.1
20E2B-3-C8-IGFR	QPAPDGFYSALMKLIGRGGVS	18.5	15.6	1.8	8.9	0.1
20E2B-2-B2-IGFR	PGTDLGFYQALRCVVIQGACD	11.7	4.9	9.0	8.1	0.1
20E2B-4-F10-IGFR	AQPCGGFYGLLEQLVGRSVCD	19.0	17.3	2.2	7.8	0.1
20E2B-4-F9-IGFR	QPDHSYFYSLLQELVGSEERL	11.9	14.7	1.9	7.7	0.1
20E2B-3-D11-IGFR	LGVTDGFYAALGYLIHGVG <u>Q</u> F	14.3	12.2	1.6	7.6	0.1
20E2B-3-C11-IGFR	CMMQDGFYAGLGCLLTAGEGR	15.3	15.4	2.1	7.5	0.1
20E2B-2-B3-IGFR	ICTG <u>Q</u> GFY <u>Q</u> VLCGLLRGTSAR	9.1	5.3	0.7	7.4	0.1
20E2B-3-D12-IGFR	QGNVLDFYGWIGRLLAKQGSD	10.3	6.2	6.0	7.3	0.1
20E2B-3-E12-IGFR	VATSQGFYSGLSELLQGGGNV	13.9	0.9	0.8	7.3	0.1
20E2B-2-B8-IGFR	IWATGDFYRLLSQLVMGRVGT	17.4	5.7	0.8	7.2	0.1
20E2B-4-G11-IGFR	RQGTGSFYLMLEQLLVGARGP	8.9	4.5	9.0	7.0	0.1
20E2B-3-D6-IGFR	DSVGDNFYQLLESLVGGHGVG	20.7	17.8	5.6	6.9	0.1
20E2B-2-B7-IGFR	LSSDGQFYRALNLLLQGSAGR	18.0	6.1	6.0	6.7	0.1
20E2B-3-C4-IGFR	ASSASGFYELLQRLAGLGLEV	23.4	20.4	3.3	6.2	0.2

		Ratios o	Ratios over Background	puno	Comparisons	18
Clone	Sequence	E-Tag	IGFR	×	IGFR/IR IR/IGFR	R/IGFR
Design	XXXXXX <u>FY</u> XX <u>h</u> XX <u>hh</u> XXXXXX	1	:	:	:	:
20E2B-3-D3-IGFR	CGSRRDFYGGIICLLGQKGVV	21.0	16.1	2.6	6.2	0.2
20E2B-4-H8-IGFR	PAGPCGFYCGLGLLLHGDQSP	7.2	5.3	6.0	5.9	0.2
20E2B-3-E9-IGFR	QAAPQDFYQGLWLLIHRDPTM	14.7	16.2	2.8	5.8	0.2
20E2B-4-H9-IGFR	RCQGTGFYTCIQELIGFGDPD	4.5	5.2	6.0	5.6	0.2
20E2B-1-A8-IGFR	TLRSPTFYDWLEMVLTHGQGG	16.1	4.4	6.0	5.0	0.2
20E2B-4-H11-IGFR	STHSRAFYDAIAQLVGSVLGP	10.7	11.0	2.3	4.8	0.2
20E2B-3-C9-IGFR	RQGGGSFYELLCGLVGGEVCV	17.9	19.7	4.2	4.6	0.2
20E2B-3-E6-IGFR	RQQASGFYRALHDLMLRTQDY	24.5	21.6	4.7	4.6	0.2
20E2B-3-E11-IGFR	SRANNLFYMGLSQLLRDNRGL	16.5	7.7	1.9	4.1	0.2
20E2B-4-G8-IGFR	GRALDPFYDQLRDLVARSGGG	11.1	14.9	3.7	4.1	0.2
20E2B-4-H10-IGFR	EASCRTFYCGLMALIGGDDQR	2.2	2.5	0.8	3.1	0.3
20E2B-3-E7-IGFR	QNGCKDFYCLIDNLIRYGPGG	14.4	8.8	3.0	3.0	0.3
20E2B-3-C12-IGFR	QHSCRTFYDCIRVLMDDGQLG	6.2	6.4	2.2	2.9	0.3
20E2B-4-G12-IGFR	LDSRRGFYDWIKALIGDRDVQ	9.6	10.0	3.8	2.6	0.4
20E2B-4-G3-IGFR	CQQKGDFYAGLVCLLRERASQ	27.2	23.8	9.1	2.6	0.4
20E2B-3-E4-IGFR	GGSQQSFYDVMCMLLQLDPTC	24.9	22.3	8.9	2.5	0.4
20E2B-3-E2-IGFR	VESDVSFYEGLMRLVWWGQGG	18.6	20.2	8.7	2.3	0.4
20E2B-2-B4-IGFR	ERAGDLFYQWFERLVAGHGLE	5.8	2.3	1.0	2.2	0.5
20E2B-3-C5-IGFR	RMPSGSFYQGIYELVTRQGGF	6.3	2.0	6.0	2.2	0.5

Clone	Common	Ratios o	Ratios over Background	puno	Comparisons	us
Design	XXXXXXFYRYFXXLLXXXXXX	E-1 ag	IGFR :	≅ ¦	IGFR/IR IR/IGFR	IR/IGFR
NNRPβ-4-G6-IR	RWPNFYGYFESLLTHFS	10.1	1.9	20.1	0.1	10 6
$NNRP\beta-4-F3-IR$	HYNAFYEYFQVLLAETW	8.6	1.3	13.6	0.1	5:01
NNRPa-2-C1-IR	EGWDFYSYFSGLLASVT	19.7	2.0	10.9	0.2	7 . 3
$NNRP\alpha-4-E1-IR$	LDRQFYRYFQDLLVGFM	11.5	6.5	21.2	0.3	3 . 5
$NNRP\alpha-3-H6-IR$	WGRSFYRYFETLLAQGI	19.1	2.1	0.9	0.3	6
$NNRP\beta-4-F7-IR$	RREGFYHYFQSLLDEYG	0.7	6.0	2.3	0.4	2.7
$NNRP\alpha-2-D1-IR$	GGGQFYRYFIDMLVLDI	18.4	1.5	3.7	0.4	2.5
NNRPα-1-A1-IR	PTGPFDRYFARRLVWRG	15.2	1.3	3.1	0.4	2.4
NNRPα-2-C10-IR	RGGAFYRYFEGLLSQHN	18.8	3.8	8.8	0.4	2.3
NNRPa-3-G1-IR	WRDPFYRYFQDLLEGER	18.9	4.2	8.6	0.5	2.1
NNRPα-4-C3-IR	WGGEFYRYFVQLLSSED	17.9	12.9	25.7	0.5	2.0
$NNRP\alpha-4-D1-IR$	GRESFYGYFLDLLQETV	16.2	12.7	23.2	0.5	1.8
NNRPβ-4-F4-IR	GHAEFYGYFQGLLDSYL	19.5	16.0	25.6	9.0	1.6
NNRPα-1-B2-IR	GGEAFYRYFWGLLTEWE	14.8	8.4	12.9	0.7	1.5
$NNRP\alpha-1-B4-IR$	LSSGFYRYFTGLLSDGQ	19.1	6.3	9.2	0.7	1.5
NNRPa-4-D9-IR	DPGAFYRYFAQLMDTWN	7.6	16.9	25.7	0.7	1.5
NNRPβ-4-F2-IR	KHEQFYEYFRNLLGAMS	21.6	20.9	30.8	0.7	1.5
$NNRP\beta-4-H12-IR$	RDGAFYRYFEDLLIAVD	5.2	13.8	20.0	0.7	
$NNRP\alpha-4-E7-IR$	RGNRFYEYFEYLLRDYG	9.4	21.9	29.7	0.7	1 . 4
$NNRP\alpha-1-B5-IR$	BLGDFYRYFQLLLADWH	14.1	5.4	7.1	8.0	1.3
$NNRP\alpha-4-C4-IR$	AQDAFYSYFSVLLGEHL	17.6	17.6	22.3	0.8	1.3
$NNRP\alpha-4-C7-IR$	IGVNFYRYFEKLLLDEF	4.5	11.2	14.9	0.8	1.3
NNRPα-4-D3-IR	TDSQFYSYFESLLETFG	16.4	13.5	17.9	0.8	1.3

Clone	Sequence	Ratios o E-Tag	Ratios over Background	ound IR	Comparisons	ns IR/ICER
Design	XXXXXXFYRYFXXLLXXXXXX	£ ;	: ;	: ;	· ·	1
NNRPβ-4-G10-IR	SSREFYSYFSGLLTTAL	8.8	8.7	11.7	0.7	1.3
NNRPβ-4-H2-IR	TGRGFYRYFEGLLEDWM	4.9	19.9	25.3	0.8	1.3
NNRPα-4-C1-IR	SGSWFYRYFEELLLQSG	15.5	18.0	21.1	0.9	1.2
NNRPα-4-C5-IR	GRGGFYQYFLDLLQTEA	18.0	23.3	26.9	6.0	1.2
NNRPα-4-C6-IR	GQNGFYRYFDTLLADWV	7.8	13.6	15.7	0.9	1.2
NNRPα-4-C12-IR	FAGSFYRYFEQLLLSEQ	12.3	16.7	19.9	0.8	1.2
$NNRP\alpha-4-D7-IR$	DPNAFYRYFEGLLWREH	10.2	23.7	27.9	0.8	1.2
$NNRP\alpha-4-D11-IR$?GLNFYRYFVGLLTDTL	5.4	19.3	22.3	6.0	1.2
NNRPβ-4-F1-IR	RHINFYGYFDDLLATWH	21.7	23.0	28.6	0.8	1.2
NNRP 3-4-F9-IR	FHRGFYRYFINLLSGDA	10.1	18.4	22.5	0.8	1.2
NNRPβ-4-F12-IR	MGSSFYRYFETLLGQGL	4.5	13.5	16.6	0.8	1.2
$NNRP\gamma-4-A3-IR$	GSLDFYSYFWERLGLGP	16.4	22.3	26.8	0.8	1.2
NNRPα-1-A7-IR	STVSFYRYFYALLQSPC	16.9	1.2	1.3	6.0	1.1
NNRPα-4-C11-IR	LGGYFYRYFEDLLNHQS	7.8	19.7	21.2	6.0	1.1
$NNRP\alpha - 4 - D8 - IR$		6.9	17.6	20.1	6.0	1.1
$NNRP\alpha-4-D10-IR$		6.4	17.2	19.5	6.0	1.1
$NNRP\alpha-4-E5-IR$	TSNWFYQYFTDLLAGED	13.2	26.1	27.6	6.0	1.1
$NNRP\alpha-4-E8-IR$	SSGGFYRYFS <u>Q</u> LLTEMN	8.7	22.9	24.2	6.0	1.1
NNRPα-4-E10-IR	VHGEFYRYFESLLRETF	3.5	12.4	13.2	6.0	1.1
NNRPβ-4-F8-IR	SDEGFYRYFAQLLYGVT	8.1	22.9	25.2	6.0	1.1
NNRPβ-4-F10-IR	ETGGFYGYFQALLATYH	5.3	17.9	19.1	0.9	1.1
NNRPβ-4-G8-IR	GDRGFYRYFEWLLNDFG	10.6	27.2	28.9	6.0	1.1
$NNRP\beta - 4 - H3 - IR$	FGGAFYRYFEALLGEMG	3.9	24.2	25.7	0.9	1.1
NNRP }-4-H9-IR	DGGAFYRYFEALLGELD	4.1	26.5	29.3	0.9	1.1
NNRP }-4-H10-IR	WHSDFYRYFLSLLQEDG	3.4	22.3	24.6	6.0	1.1
NNRPy-4-A6-IR	EEEGFYGYFYRLLGVER	14.9	25.8	27.6	0.9	1.1
NNRPy-4-A8-IR	MDAGFYGYFSDLLANWG	8.6	22.8	24.7	6.0	1.1

FIGURE 2P (Con't)

Clone Design	Sequence XXXXXXFYRYFXXLLXXXXXX	E-Tag	IGFR	H	IGFR/IR IR/IGFR	R/IGFR
NNRPy-4-A10-IR	SGFAFYQYFQELLAGHD	7.6	20.3	22.0	6.0	1.1
NNRPy-4-B6-IR	GDGGFYGYFASLLSGEG	12.2	22.3	24.2	0.9	1.1
$NNRP\gamma$ -4-B9-IR	EANGFYRYFYDLLQDFG	6.7	22.9	25.9	6.0	1.1
NNRPa-4-C8-IR	AVNGFYRYFNRLLESVE	8.5	16.3	16.0	1.0	1.0
NNRPα-4-C9-IR	QQDGFYRYFLDLLDEVA	5.6	20.7	19.9	1.0	1.0
$NNRP\alpha-4-C10-IR$	ISQGFYGYFSRLLQDTE	6.7	16.5	17.2	1.0	1.0
NNRPa-4-E11-IR	YSTGFYRYFLDLLDGMP	6.0	20.3	20.9	1.0	1.0
NNRP(3-4-F11-IR	PNGDFYRYFLDLLGSVG	7.7	21.8	21.9	1.0	1.0
NNRPβ-4-G2-IR	RHQAFYSYFRDLPRECP	19.1	24.7	25.6	1.0	1.0
NNRPβ-4-G9-IR	ETEGFYRYFEELLAQVA	7.8	27.3	26.4	1.0	1.0
NNRPB-4-H7-IR	AGDRFYDYFDRLLADYD	2.6	26.6	27.9	1.0	1.0
NNRP()-4-H8-IR	GGSGFYRYFWGLLAEQE	3.6	23.0	24.1	1.0	1.0
NNRPy-4-B1-IR	LLNRLYRYFAGAEGWFG	17.6	24.5	23.4	1.0	1.0
NNRPy-4-B10-IR	DGSGFYRYFEMLLGSGL	5.5	18.3	19.0	1.0	1.0
$NNRP\alpha-1-B3-IR$	RDMAFYRYFSHLLESFQ	16.4	13.4	12.7	1.1	6.0
$NNRP\alpha-2-C2-IR$	GNAGFYRISRILWQGTE	22.5	24.4	21.3	1.1	6.0
NNRPα-2-C3-IR	GNAGFYRYFADLMAGYE	19.6	21.7	19.7	1.1	6.0
NNRPα-2-D10-IR	YQAAFYRYFATLLSTTD	17.8	6.3	5.4	1.2	6.0
$NNRP\alpha-3-E11-IR$	GGLGFYRYF <u>Q</u> LLLGSSG	12.9	10.8	9.6	1.1	6.0
NNRPa-3-F5-IR	DGSGFYGYFDFVLRQFE	25.1	18.3	17.0	1.1	6.0
NNRPa-3-F8-IR	VGSGFYRYFDQLLGMYG	22.2	15.7	13.9	1.1	6.0
$NNRP\alpha-3-F10-IR$	YGTDFYLYFDQLLLQYG	20.5	14.6	13.1	1.1	6.0
NNRPa-3-G7-IR	FNSSFYLYFRDLLNTVG	21.0	18.3	15.6	1.2	0.9
NNRPa-4-C2-IR	RAAGFYRYFEDLLGARG	25.5	25.1	23.3	1.1	6.0
NNRPα-4-D12-IR	TGAGFYRYFIDLLGETG	14.7	19.7	18.5	1.1	0.9
NNRPβ-4-G3-IR	RDLEFYGYF <u>Q</u> BLLRLNF	14.6	27.8	25.7	1.1	6.0
NNRP }-4-G4-IR	GMGPFYRYFIDLLRESD	20.0	28.6	24.9	1.1	6.0
NNRPy-4-A5-IR	HGDGFYQYFMEVLRLQN	17.0	29.0	27.3	1.1	6.0

FIGURE 2P (Con't)

Clone	Sequence	E-Tag	IGFR	IR	IGFR/IR IR/IGFR	WIGFR
Design	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX	1	1	:	1	1
$NNRP\gamma-4-A12-IR$	AFYRYFRDLLFSGF	4.9	16.3	14.9	1.1	6.0
NNRPy-4-B8-IR	DDRGFYRYFESLLLGSS	6.1	21.3	19.9	1.1	6.0
$NNRP\alpha-1-A5-IR$	LSTSFYQYLAGLLRGDR	2.3	1.4	1.1	1.2	0.8
NNRPa-1-B7-IR	GSSGFYRYFNMLMLSQT	19.2	15.7	12.4	1.3	0.8
$NNRP\alpha-2-C7-IR$	GDRGFYRYFEGLLASVG	19.6	20.0	16.5	1.2	0.8
NNRPα-2-C11-IR	NSAAFYRYFEQLLEREV	20.1	20.0	16.3	1.2	0.8
NNRPa-2-C12-IR	LSDGFYRYFEQLMGARS	14.3	10.1	8.5	1.2	0.8
NNRPa-2-D12-IR	RSTLFYRYFQNLLEEVG	11.5	11.4	9.3	1.2	0.8
NNRPα-3-G2-IR	TRGGFYRYFEDLLQVYS	20.8	20.7	16.1	1.3	0.8
$NNRP\alpha - 3 - G8 - IR$	GVSGFYRYF <u>Q</u> SLLDSYG	14.7	11.0	9.5	1.2	0.8
NNRPα-3-G10-IR	QNDAFYSYFNSLLQAYT	18.8	16.5	13.9	1.2	0.8
$NNRP\alpha - 3 - G11 - IR$	RQQDFYRYFRQLLLEEV	12.0	10.3	8.5	1.2	0.8
NNRPα-3-G12-IR	EGSGFYRYFEKLLL <u>o</u> sp	11.7	11.8	9.3	1.3	0.8
NNRPy-4-B2-IR	RHKAFYRYFEELLQKNV	22.8	30.3	25.3	1.2	0.8
$NNRP\alpha-1-B8-IR$	GRMTRLIVRSTVISRELLHYSL	16.1	10.1	6.9	1.5	0.7
NNRPa-2-C5-IR	QALSFYRYFERLLDEVS	18.1	19.2	13.7	1.4	0.7
NNRPα-2-C9-IR	SKSAFYRYFDELLGNSG	22.9	21.7	16.1	1.3	0.7
$NNRP\alpha-2-D2-IR$	LGGAFYRYFAQLLNSHV	26.1	26.3	17.6	1.5	0.7
$NNRP\alpha-2-D5-IR$	LNSGFYGYFV <u>Q</u> LLSGH <u>Q</u>	21.7	21.1	15.4	1.4	0.7
$NNRP\alpha-2-D11-IR$	SQSSFYRYFESLLEDNP	12.3	10.8	7.8	1.4	0.7
$NNRP\alpha - 3 - E2 - IR$	ADGGFYGYFAALLGSVS	24.4	25.5	18.3	1.4	0.7
$NNRP\alpha - 3 - E4 - IR$	QNGSFYRYFIALLGDSG	23.0	22.3	14.7	1.5	0.7
$NNRP\alpha - 3 - F4 - IR$	WDTGFYRYFIELLEDRD	24.9	25.1	17.6	1.4	0.7
$NNRP\alpha - 3 - G4 - IR$	HPRDFYRYFERLLNQVD	20.9	20.4	14.1	1.5	0.7
NNRPα-3-H4-IR	DGGAFYRYFMDLLGAHE	17.7	17.6	11.6	1.5	0.7
NNRPα-4-E12-IR	AGRGFYRYFEHLLAGRE	4.3	15.4	10.8	1.4	0.7
NNRPβ-4-G11-IR	SSRGFYRYFRELLADSW	9.9	18.4	13.1	1.4	0.7
$NNRP\beta-4-H6-IR$	KYSGFYEYFNALLGRRE	2.2	16.1	11.7	1.4	0.7

FIGURE 2P (Con't)

Clone	Sequence	E-Tag	IGFR	IR I	IGFR/IR IR/IGFR	R/IGFR
Design	XXXXXXFYRYFXXLLXXXXXX	i i	1	1	1	1
$NNRP\beta-4-H11-IR$	DYTAFYGYFNNLLRTSG	2.3	12.4	9.0	1.4	0.7
$NNRP\alpha-1-B1-IR$	FQSSFYGYFESLLMSYK	18.8	18.7	11.5	1.6	9.0
NNRPa-2-D7-IR	DINAFYRYFEGLLWSEH	21.0	21.8	13.2	1.6	9.0
$NNRP\alpha-2-D8-IR$	GGSSFYRYFEQLLAQWE	20.2	19.8	12.2	1.6	9.0
$NNRP\alpha-3-E1-IR$	SQGGFYRYFEKLLDEVT	20.0	20.5	12.9	1.6	9.0
NNRPa-3-E5-IR	RSGLFYRYFEELLQGAI	20.0	24.5	15.5	1.6	9.0
NNRPa-3-H3-IR	QGGGFYHYFLSLLEEVG	19.8	19.1	12.2	1.6	9.0
NNRPa-3-H5-IR	WRGAFYRYFQTLLSDEG	19.9	18.0	11.1	1.6	9.0
$NNRP\alpha-1-A3-IR$	AAGFYGYFYSLLGDQT	24.4	14.9	7.9	1.9	0.5
$NNRP\alpha-3-E6-IR$	RNSGFYRYFQHLVSEWE	23.1	19.0	9.6	2.0	0.5
NNRPQ-3-F9-IR	QHRLFYSYFAELLGRDT	21.1	18.8	9.6	1.9	0.5
$NNRP\alpha-3-G6-IR$	QIDEFYRYFADQLRGFA	22.4	17.7	9.0	2.0	0.5
$NNRP\alpha-3-H9-IR$	LGGGFYRYFNLLVMGSG	18.3	13.1	6.9	1.9	0.5
$NNRP\alpha-1-A8-IR$	GDRAFYRYFQRQLEGWG	16.9	13.8	5.7	2.4	0.4
NNRP0-1-A9-IR	CEDAFYRYFVNLLGQGC	16.5	15.2	5.6	2.7	0.4
NNRPa-2-D6-IR	NYSQFYRYFEMLLEGDV	19.4	18.5	6.8	2.7	0.4
$NNRP\alpha-3-F6-IR$	VGDAFYRYFQGLLRQDQ	22.8	19.5	7.9	2.5	0.4
NNRPa-3-H2-IR	MHGSFYRYFQDLLQAPP	19.9	18.9	8.5	2.2	0.4
$NNRP\alpha-1-B6-IR$	DVGDFYRYFGLLLTSDR	14.1	11.5	3.9	3.0	0.3
NNRPa-2-C6-IR	NSAAFYGYFSQLLAQIR	18.4	19.2	4.1	4.7	0.2
NNRPy-4-A1-IR	IIGGFYSYFNSVLRLGT	9.7	10.9	1.8	6.0	0.2
NNRPy-4-A7-IR	RFDPFYSYFVNLLGASA	2.5	6.3	1.3	4.9	0.2
NNRPy-4-A9-IR	EGSGFYGYFFSLLGLQG	3.0	10.0	1.4	7.1	0.1
$NNRP\gamma-4-B11-IR$	LKDGFYDYFWQRLHLGS	4.1	18.7	1.2	15.5	0.1

		Ratios ov	Ratios over Background	pun	Comparisons	isons
Clone	Sequence	E-Tag	GFSR IR I		IGFR/IR	IR/IGFR
Design	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX	1	:	1	;	!
R20-4-F9-IGFR	PLAELWAYFEHSEQGRSSAH	33.1	19.3	1.0	19.3 0.1	0.1
R20-4-H4-IGFR	PVLSGLLRYFAGGPLGQPQS	24.1	5.6	3.2	1.8	9.0
R20-4-F9-IGFR	GGYLDDLWHYFRDGQALQPW	2.5	2.4	1.4	1.7	0.6
R20-4-D6-IGFR	VDQRQGGWLLALENYFRSTV	6.1		1.9	1.5	0.7
R20-4-G2-IGFR	DVPAGGLLRQMWVYFRDSDP	6.3		2.0	1.1	6.0

ξ	ç	Ratios ov	Ratios over Background	pun	Comparisons	1S
Cione	Sequence	E-Tag	E-Tag IGFSR IR	¥	IGFR/IR IR/IGFR	GFR
Design	XXXXCXXXXXXXXXXXXXXXXX	1	;	:		
20C-3-F3-IGFR	RRVACTQADGLLCESDPLKALLSYF	35.5	35.5 32.8 17.9 1.8	17.9	1.8	0.5

		Ratios over	Ratios over Background	pun	Comparisons	isons	
Clone	Sequence	E-Tag	IGFSR	IR	IGFR/IR	IR/IGFR	
Design	XXXLXXLXXYFXXXXXX	:	1 1	1	;	!	
rB6-4-E7-IR	LDPLDALLQYFWSVPGH	26.4	1.0	15.5	0.1	15.5	
rB6-4-A12-IR	LDALDRLMRYFEERPSL	34.9	1.0	12.0	0.1	12.0	
rB6-3-E6-IR	ADELEWLLDYFMHQPRP	9.0	1.0	4.8	0.2	4.8	
rB6-4-E11-IR	DQELGWLRGYFEWTARD	31.2	1.6	5.9	0.3	3.7	
rB6-4-F12-IR	DGVLEELFSYFSATVGP	30.4	1.0	3.4	0.3	3.4	
rB6-4-D11-IR	PMNLSELWDYFRLKPGR	41.9	15.7	30.2	0.5	1.9	
rB6-4-A8-IR	DSILRELRDYFAPYSHC	25.6	2.4	4.6	0.5	. 1.9	
rB6-4-E8-IR	DDALEWLLNYFQNGHVQ	33.0	9.7	15.9	9.0	1.6	
rB6-4-B9-IR	GDILDALLRYFEFGVDT	42.7	17.2	21.7	0.8	1.3	
rB6-3-A6-IR	GDQLAWLLAYFQSDGSD	32.3	5.9	2.8	1.0	1.0	
rB6-4-C7-IR	DGVLEGLLSYFTSTNSH	31.4	5.6	2.3	1.1	6.0	
rB6-4-H12-IR	ARPLDWLLDYFKQGARG	26.0	10.0	7.2	1.4	0.7	
rB6-3-C6-IR	DDMLRQLWLYFEASAGG	34.2	19.1	12.8	1.5	0.7	
rB6-4-G12-IR	DPWLAWLGRYFGETATG	37.7	6.1	3.1	2.0	0.5	
rB6-4-G12-IR	DPTLFGLLRYFQESGIA	33.3	7.6	3.5	2.2	0.5	
rB6-4-C11-IR	MDPLRGLLMYFSQGGLV	26.6	18.7	4.7	4.0	0.3	
rB6-4-G8-IR	DGLLWQLWDYFALSEHR	37.3	7.4	1.3	5.7	0.2	
rB6-4-B8-IR	DNWLSALMAYFMGSGES	31.1	28.6	1.0	28.6	<0.1	
rB6-4-D7-IR	DDVLNYLLGYFRQSDGL	24.1	29.4	1.0	29.4	<0.1	

		Ratios over	Ratios over Background	pun	Comparisons	risons	
Clone	Sequence	E-Tag	IGFSR	IR	IGFR/IR	IR/IGFR	
Design	XXXLXXLXXXXXXX	1	;	;	:	1	
rB6-4-B10-IGFR	RPVLGWLFDYFVASDPM	33.1	26.9	1.0	26.9	<0.1	
rB6-3-E6-IGFR	RWPLSALMDYFRRSDGV	37.6	26.6	1.0	26.6	<0.1	
rB6-4-B9-IGFR	DGVLASLWRYFVSGGTL	39.2	26.3	1.0	26.3	<0.1	
rB6-3-F5-IGFR	DRQLGWLWDYFHLTDLP	33.2	15.6	1.0	15.6	0.1	
rB6-3-B6-IGFR	DGILGLLMAYFVES?RV	37.4	13.3	1.0	13.3	0.1	
rB6-3-D4-IGFR	QDLLGRLWLYFAETDTV	31.2	20.7	2.0	10.4	0.1	
rB6-4-D10-IGFR	SGVLADLFRYFQRHPWP	31.7	10.1	1.0	10.1	0.1	
rB6-3-D6-IGFR	DPPLGGLWTYFSRSDPG	33.9	6.6	1.0	6.6	0.1	
rB6-4-F9-IGFR	DSVLRSLYSYFASGDIA	34.3	28.3	3.0	9.4	0.1	
rB6-3-E1-IGFR	DGVLAALEAYFRHGPRD	30.5	9.3	1.0	9.3	0.1	
rB6-3-B2-IGFR	DEILGALYSYFSLSGGA	22.2	8.8	1.0	8.8	0.1	
rB6-3-D7-IGFR	QDVLGALQRYFASGEPW	31.2	7.6	1.0	7.6	0.1	
rB6-4-C11-IGFR	DSVLQYLLNHFGADSKQ	33.7	7.6	1.0	7.6	0.1	
rB6-4-F12-IGFR	NEVLEGLFSYFVY? ANG	38.1	7.3	1.0	7.3	0.1	
rB6-4-F7-IGFR	SGILGQLLRYFKGAGGG	38.6	7.3	1.0	7.3	0.1	
rB6-3-G6-IGFR	DELLDRLWQYFQVGGDL	34.0	7.1	1.0	7.1	0.1	
rB6-4-E8-IGFR	PGILLDLWRYFASAPDQ	37.6	6.9	1.0	6.9	0.1	
rB6-4-G10-IGFR	DSVLLDLYEYFSSGSSG	34.9	14.5	2.2	9.9	0.2	
rB6-4-B12-IGFR	DGMLSRLWEYFAGTNVP	36.3	28.9	4.5	6.4	0.2	
rB6-3-B5-IGFR	DVILGGLWDYFASGGGH	17.2	6.1	1.0	6.1	0.2	
rB6-3-C5-IGFR	GGVLAALERYFRVSAGD	38.7	15.8	2.9	5.4	0.2	
rB6-4-B8-IGFR	DEVLGRLWAYFAQESLG	31.9	22.0	4.1	5.4	0.2	
rB6-3-H2-IGFR	DGILQSLWDYFARSPVG	31.8	22.4	4.2	5.3	0.2	
rB6-3-E5-IGFR	VDILSELWDYFRRGEEG	37.0	20.5	4.0	5.1	0.2	
rB6-3-B3-IGFR	DKVLRLLGEYFATHSKG	31.7	4.8	1.0	4.8	0.2	
rB6-4-G7-IGFR	QGPLAWLRDYFASGTRS	37.4	10.0	2.1	4.8	0.5	
rB6-3-A3-IGFR	QDVLRSLLSYFMGNGDV	27.2	4.7	1.0	4.7	0.2	
rB6-4-E9-IGFR	DGVLSKLWEYFKIQGND	37.3	20.1	4.8	4.2	.0.2	

Figure 3D

		Ratios ove	Ratios over Background	pun	Comparisons	sons
Clone	Sequence	E-Tag	IGFsR	꼼	IGFR/IR IR/IGFR	R/IGFR
Design	XXXLXXLXXYFXXXXXX	i i	:	1	1	1
rB6-3-F1-IGFR	NTILGDLWRYFAGSGGM	26.5	5.8	1.4	4.1	0.2
rB6-4-B7-IGFR	?DVLKKL?VYFELSGGA	31.1	11.4	2.9	3.9	0.3
rB6-4-C10-IGFR	GGPLQGLYTYFKQSPVC	32.2	3.7	1.0	3.7	0.3
rB6-3-A1-IGFR	DRLLSGLWAYFAGNGGS	21.1	3.5	1.0	3.5	0.3
rB6-3-F6-IGFR	DLILQSLLDYFQGRPVG	25.1	3.5	1.0	3.5	0.3
rB6-3-H5-IGFR	LALLPMLWDYFVATDPQ	35.5	18.1	5.6	3.2	0.3
rB6-4-D8-IGFR	$\overline{\text{DSILRELRDYFARTHIA}}$	36.2	22.5	7.5	3.0	0.3
rB6-4-A8-IGFR	DGVLGQLWQYFAQYPGS	41.1	30.6	10.6	2.9	0.3
rB6-4-H8-IGFR	PPLDALWEYFTGTARD	38.7	33.0	11.5	2.9	0.3
rB6-3-E2-IGFR	DNVLEGLWSYFALWSQL	20.9	2.2	1.0	2.2	0.5
rB6-3-C2-IGFR	SAVLEYLLAYFARTGĀA	31.0	2.1	1.0	2.1	0.5
rB6-4-G8-IGFR	DRALGPLWRYFMVNNGQ	38.7	5.5	2.6	2.1	0.5
rB6-3-G5-IGFR	WRILDRLLAYFKESQGD	32.8	2.0	1.0	2.0	0.5
rB6-4-C9-IGFR	DDVLVTLFQYFRASTGV	37.6	30.2	15.1	2.0	0.5
rB6-4-D11-IGFR	FDVLTWLGRYF*MNTGK	36.6	5.5	3.0	1.8	0.5
rB6-4-B11-IGFR	RDVLDGLREYFRASVGG	25.2	4.2	2.4	1.8	9.0
rB6-4-E11-IGFR	IKTLNDLLAYFRGDLDV	38.1	29.8	22.2	1.3	0.7
rB6-3-G3-IGFR	DEALLWLMRYFRGSPSP	31.6	8.7	7.2	1.2	0.8
rB6-4-H12-IGFR	ESPLDALRAYFSGRRNW	40.1	2.8	2.5	1.1	6.0
rB6-4-G12-IGFR	IQSL*DLLQYFVSSPSV	36.7	32.5	31.4	1.0	1.0
rB6-3-C4-IGFR	GGILD?LQDYFRSTDVG	37.1	6.2	13.5	0.5	2.2

Comparisons IGFR/IR IR/IGFR 7
Ratios over Background Comparisons E-Tag IGFsR IR IGFR/IR IR/IGFR
Sequence xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx
Clone Design R20β-4-F8-IR

		Ratios ove	Ratios over Background	pun	Comparisons	isons	
Clone	Sequence	E-Tag	IGFSR	IR	IGFR/IR	IR/IGFR	
Design	HLCVLEELFWGASLFGYCSG	;		!	1 1	1	
F815-4-H9-IR	PLCVLEELFWSTPLFGQCSY	34.9	6.0	37.6	<0.1	40.8	
F815-3-B1-IR	HLCVLEELFWGASLFAQCVG	31.7	6.0	35.8	<0.1	39.3	
F815-3-D1-IR	DLCVLEELFWGASRFGQCSG	30.4	6.0	33.5	<0.1	38.9	
F815-3-D4-IR	HLCVLEELFWGASLFGQCAG	31.5	0.9	33.6	<0.1	38.8	
F815-3-C5-IR	HLCVVEELFWGASLFGQCSG	31.1	0.8	31.2	<0.1	38.5	
F815-4-H3-IR	NLCDLEVLFWGASLFRQCSG	33.7	1.0	37.2	<0.1	38.4	
F815-3-A5-IR	PLCVLEEQFWGASLFGQCSG	37.4	1.1	40.9	<0.1	38.3	
F815-3-D7-IR	QLCVLEELFWGASEFGQCSG	33.6	6.0	34.3	<0.1	38.3	
F815-3-A1-IR	HLCELEELFWGASLFGQCSG	29.8	6.0	34.8	<0.1	38.0	
F815-4-H4-IR	PLCVLEELFWGESLFGQCSG	31.1	0.9	32.7	<0.1	38.0	
S	HLCVLEELFWGASRFGQCSG	32.8	1.0	39.1	<0.1	37.9	
F815-3-B3-IR	KLCVLEELFWGASLFGQCSG	33.7	1.0	37.5	<0.1	37.5	
F815-3-A4-IR	YLCVLEELSWGASLFGQCSG	32.5	1.0	36.9	<0.1	37.5	
F815-3-D2-IR	HLCVLEELLWGASLFAQCSG	31.9	6.0	34.1	<0.1	37.4	
F815-3-C4-IR	<u> </u>	31.6	0.8	31.8	<0.1	37.4	
-3-B4	HLCVLEELFWGGNLFSQCSG	33.8	1.0	36.7	<0.1	37.3	
F815-3-C1-IR	HLCVLEELFWGASLYGQCSG	29.0	6.0	35.0	<0.1	37.3	
F815-4-G9-IR	SLCALEEQFWGAALFGYCSG	36.5	1.0	38,9	<0.1	37.1	
F815-4-G6-IR	HLCVLEEQFWGASLFDGCAG	34.9	1.0	36.4	<0.1	37.0	
5-3	QLCVLEELFWGASLFGQCSG	34.7	1.1	39.3	<0.1	36.9	
F815-4-G5-IR	PLCVLEELFWGAALFGQCSG	26.5	1.0	35.1	<0.1	36.8	
F815-3-B5-IR	HLCVLEELFWGASLFGQCTG	33.2	6.0	34.1	<0.1	36.8	
F815-4-F4-IR	PLCVLEELFWGGSLFGQCSG	28.6	0.8	30.0	<0.1	36.7	
F815-3-A2-IR	QLCVLEELVWGASLFGQCSG	32.5	1.0	36.6	<0.1	36.6	
F815-3-B6-IR	HLCVVEELIWGASLFGQCSR	31.6	6.0	32.9	<0.1	36.5	
F815-4-H7-IR	DLCVLEELFWGASLFGQCAG	33.7	1.0	37.6	<0.1	36.4	
F815-4-H8-IR	QLCVLEERFWGASLFGQCSG	35.8	1.0	37.0	<0.1	36.4	
F815-4-G7-IR	NLCVLEELFWGAALFG <u>Q</u> CSG	33.7	1.0	35.8	<0.1	36.3	

Figure 4B

Clone	Sequence	Ratios ov E-Tag	Ratios over Background E-Tag IGFsR II	und IR	Comparisons IGFR/IR IR/IC	risons JR/IGFR
Design	HLCVLEELFWGASLFGYCSG	D 1	1	; ;		; ; ; ; ;
F815-3-A6-IR	QLCVLEELFWGSSLFGQCSG	34.6	1.1	39.0	<0.1	36.2
F815-3-D3-IR	DLCVVEELFWGKSLFGQCSG	33.8	1.0	36.2	<0.1	36.2
F815-3-B12-IR	DLCVLEELFWGSSLFGQCSG	33.2	1.0	35.7	<0.1	36.2
F815-4-G10-IR	YLCVLEEQFWGASLFRQCFG	35.4	1.0	37.2	<0.1	36.1
F815-4-E3-IR	HLCVLEELLWGSSLFGQCSG	32.4	1.0	35.0	<0.1	36.1
F815-4-E6-IR	PLCGLEELFWGASLFGQCSD	33.2	1.0	34.5	<0.1	36.1
F815-4-F1-IR	HLCVLEELFWGSSLFAQCSG	29.4	6.0	32.5	<0.1	36.0
F815-4-G8-IR	PLCAIEELFWGAALFGQCSG	36.8	1.1	38.2	<0.1	35.9
F815-4-H12-IR	HLCVLEEQFWGASLFGDCSG	30.5	6.0	31.9	<0.1	35.9
F815-4-G3-IR	PLCVLEELFWGAPLFGQCSD	31.4	1.0	35.7	<0.1	35.7
F815-3-C2-IR	DLCGLEELFWGAALFGQCTS	32.3	1.0	36.1	<0.1	35.6
F815-4-E10-IR	QLCVLEKQLWGASLFWQCSG	35.4	1.0	36.5	<0.1	35.4
F815-3-A12-IR	HLCVLEELFWGASLYGQCPG	32.1	1.0	36.3	<0.1	35.3
F815-3-B8-IR	HLCVLEELFWGASLFDQCSG	33.6	1.0	35.8	<0.1	35.3
F815-3-B2-IR	HLCVLEELLWGASLFGQCSG	31.0	1.0	35.3	<0.1	35.3
F815-3-C3-IR	PLCVLEELFWGVSLFGQCGG	30.1	1.0	35.3	<0.1	35.3
F815-3-A7-IR	HLCVLEELFWGASQWGQCSG	33.1	1.0	35.8	<0.1	35.2
F815-4-F9-IR	RLCVLEEQFWGGALFGQCSG	33.4	1.0	35.7	<0.1	35.2
F815-3-B7-IR	QLCVLEELFWGVSLFAQCSG	32.0	1.0	33.5	<0.1	35.0
F815-4-E4-IR	HLCVLEELFWGAALFGQCFG	28.0	1.0	33.4	<0.1	35.0
F815-4-E12-IR	YLCVLEELFWGASQFGQCSG	28.0	6.0	30.2	<0.1	34.8
F815-4-F8-IR	HLCVLEELYWGASLFGQCSG	33.8	1.0	35.2	<0.1	34.7
F815-3C7-IR	HLCVLEERFWGVSLFGQCSG	33.9	1.0	34.7	<0.1	34.7
F815-4-F10-IR	PLCVLEELFWGASRFGQCSG	32.7	1.0	34.2	<0.1	34.7
F815-3-D11-IR	HLCVLEDLFWGASLFDQCSG	35.4	1.1	37.3	<0.1	34.6
F815-4-E7-IR	HLCDLEVLFWGASLFGQCSG	30.3	6.0	32.2	<0.1	34.6
F815-3-A10-IR	QLCILEEQFWGTSLFGYCSG	34.0	1.1	36.4	<0.1	34.3
F815-3-B11-IR	ALCVLEELFWGESLFG <u>Q</u> CSG	33.7	1.1	36.3	<0.1	34.2

Figure 4B (Con't)

		Ratios ov	Ratios over Background	pun	Comparisons	risons
Clone	Sequence	E-Tag	IGFSR	K	IGFR/IR	IR/IGFR
Design	HLCVLEELFWGASLFGYCSG	;	1	1	3 1	1 ;
F815-4-F11-IR	RLCVLEERFWGAALFGQCSG	31.8	1.0	33.7	<0.1	34.2
F815-3-A9-IR	PLCVLEELFWGASLFGQCSG	31.9	1.0	35.5	<0.1	34.1
F815-4-G11-IR	SLCVLEELFWGGSRFGQCSG	32.3	1.0	34.4	<0.1	33.9
F815-3-D8-IR	HLCLLEEQFWGASLFGYCFE	32.3	1.0	33.3	<0.1	33.7
F815-4-G4-IR	HLCVLEEQFWGASLFGQCSG	23.8	1.0	32.2	<0.1	33.7
F815-3-C8-IR	DLCLLEELLWGASRFGQCSG	33.9	1.0	35.1	<0.1	33.6
F815-4-G12-IR	YLCVLEERFWGASLFGQCSG	31.7	1.0	33.5	<0.1	33.5
F815-3-D12-IR	HLCVLEEQFWGASLFGSCSG	33.3	1.0	34.8	<0.1	33.4
F815-4-F7-IR	QLCVLEEQLWGASLFGQCSG	33.3	1.0	34.3	<0.1	33.4
F815-4-F2-IR	HLCVLEELF*GESLFGYCSG	26.1	1.0	33.8	<0.1	33.3
F815-3-B9-IR	HLCVLEELFWGASLFGQCSG	33.6	1.1	35.7	<0.1	33.2
F815-4-H2-IR	PLCVLEELFWGASHFGQCSG	36.1	1.2	38.4	<0.1	33.0
F815-4-E11-IR	HLCVLEELVWGASLFGQCAG	33.2	1.1	35.4	<0.1	33.0
F815-4-G1-IR	QLCVLEELIWGASLFGQCAG	27.9	1.0	31.5	<0.1	32.8
F815-3-A11-IR	HLCGLEELFWGASLFGQCSG	37.7	1.2	40.1	<0.1	32.7
F815-4-F6-IR	HLCVLEELVWGESLFGQCSG	32.3	1.1	34.6	<0.1	32.6
F815-3-D9-IR	RLCVLEELYWGASLFGQCSG	31.4	1.0	32.5	<0.1	32.5
F815-3-C11-IR	RLCILEELFWGASLFGQCSG	33.4	1.1	35.7	<0.1	31.9
F815-4-G2-IR	HLCVLEELFWGATLFDQCSG	30.2	1.1	34.3	<0.1	31.4
F815-3-C9-IR	HLCFLEELFWGASMFGQCSG	29.7	1.0	31.4	<0.1	31.0
F815-4-H10-IR	HLCIVEELFWAAPLFGQCSG	31.9	6.0	27.6	<0.1	29.4
F815-4-F3-IR	HLCVLEELWWGASLFAQCSA	19.4	1.0	28.0	<0.1	28.9
F815-4-F5-IR	NLCALEELFWGASQFRYCPG	12.3	6.0	24.8	<0.1	26.8
F815-4-H1-IR	RLCVLEELFWGASIFGQCSG	6.9	1.0	15.8	0.1	16.5
F815-4-E5-IR	PLCVLEELFWGASLFGQCPG	3.5	1.0	13.6	0.1	14.0
F815-4-H5-IR	NLCVLEELFWGASLFGQCSG	5.5	1.0	13.1	0.1	13.5
F815-3-C10-IR	QLCVLG#RFWGGSLCGYCSD	3.5	1.1	5.2	0.2	4.5

Figure 4B (Con't)

Ratios over Background Comparisons E-Tag IGFSR IR IGFR/IR IR/IGFR	39.1 1.8 2/./ U.1 15.1 33.4 12.3 1.0 12.3 0.1		8.5 1.0 8.5	35.2 23.9 4.8 5.0 0.2	33.9 4.8 1.0 4.8 0.2					30 4 9.0 5.0 1.8 0.6	30.8 3.7 2.2 1.7 0.6	7 6 1.0 2.0 0.5 2.0	20.5 1.0 2.0 0.5 2.0	7.6 1.0 2.5 0.4 2.5	18,4 1.0 6.8 0.1 6.8	
	SEQUENCE HUCVLEELFWGASLFGYCSG	PLCFLQELFGGASLGGYCSG	FMCGLQELVGGAALLGHCSG	PLCFLQELFGGGSLSGYCSG	FLCGLEELAWGVSRSGYCFG	PLCFLAELFSGSALGGDCSR	PLCVLQELFGGGSLGGYCSG	QLCVLE#LFWGACLFGYCAG	PLCGLQELSGVASLFGQCSG	RVCVLEQLVWGASLFGA*SG	FYCGLEELSWGAALFGYCSG	FLCGLEELSQGAVLFGHCYG	HLCVLVGLFWDASLFGQCSG	QRCIRAALFWCATLLGGCAG	HOCIPDGMSQGAALRGNCSD	HLCVLEDELWGVSLFGYCSS
	Clone Parental/Design						~					~			F815-4-G12-IGFR	F815-3-H1-IGFR

		Ratios ove	Ratios over Background	pun	Comparisons	sous
Clone	Sequence	E-Tag	IGFSR	~	IGFR/IR I	IR/IGFR
Parental/Design	HLCVLEELFWGASLFGYCSG	39.1	1.8	27.7	0.1	15.4
F820-4-B5-IR	HLCMLEEQFWGASLFSRCSG	28.1	6.0	17.9	<0.1	21.1
F820-4-A2-IR	TCAFWKNGSGVRRCSVTAVV	34.0	1.6	22.7	0.1	13.9
F820-4-E2-IR	PLCGLKN.SGVRLCSSPALV	21.3	0.7	9.0	0.1	13.4
F820-4-D10-IR	PLCLQEELFWGASLFGYCSG	34.1	1.0	12.1	0.1	12.1
F820-4-H7-IR	PLCDLEELFWGASLFGDCPG	14.2	9.0	6.5	0.1	11.6
F820-4-G6-IR	DLCVLEELFWDGSLFASCSG	14.0	0.5	6.1	0.1	11.5
F820-4-C2-IR	PLCVLEEQLWGTALFGSCTG	38.1	1.2	11.8	0.1	6.6
F820-4-B4-IR	PLCLVEELLWGASLFSQCTG	15.1	0.7	6.4	0.1	8.7
F820-4-C7-IR	PLCDLEELYWGAALFGSCSG	46.3	2.7	22.2	0.1	8.2
F820-4~F10-IR	GLCFLEEQFWGTSLFRDCPG	14.5	9.0	4.7	0.1	8.0
F820-4-G5-IR	PLCVVEELFWGASLYGQCSG	8.8	9.0	4.4	0.1	7.5
F820-4-F2-IR	RLCVLEELFWGASRFRGCSG	11.7	9.0	4.2	0.1	7.4
F820-4-H8-IR	PLCVLEELHWGAALFGYCSG	16.0	9.0	4.7	0.1	7.3
F820-4-D7-IR	NLCVVEELFWGASLFPNCSG	14.5	0.8	5,9	0.1	7.1
F820-4-B2-IR	QLCVLEELFWGASMFEDCSG	5.0	0.4	2.4	0.2	6.9
F820-4-C3-IR	HLCVLEEQFWGASLFGQCSG	37.5	1.1	7.5	0.2	9.9
F820-4-H4-IR	PLCVLEEI YWGAALFGDCYG	21.2	1.1	6.4	0.2	5.9
F820-4-B10-IR	PLCVLEELFWGLSLDKNCS	7.5	0.7	3.7	0.2	5.6
F820-4-A5-IR	QLCVLEELFWGASLFSGCSG	5.3	0.8	4.4	0.2	5.2
F820-4-F6-IR	PLCDLEALFWGESLFGGCSG	5.7	9.0	3.0	0.2	4.9
F820-4-F1-IR	HLCVLEEMFWGTSHFDGCSG	9.1	1.0	4.7	0.2	4.7
F820-4-A3-IR	DLCVLEELFWGAPLFGLCSG	5.9	0.8	3.5	0.2	4.5
F820-4-D1-IR	DLCVLEELFWGVALYGGCSG	25.7	2.3	10.5	0.2	4.5
F820-4-F5-IR	QLCVLEELYWGASIFGHCSG	3.7	9.0	2.7	0.5	4.2
F820-4-F12-IR	HLCVLEDRFWGASIFGPCSG	11.3	9.0	2.2	0.3	3.5
F820-4-A11-IR	HLCGMEEMFWGVALFRNCSG	7.6	0.8	2.7	0.3	3.5
F820-4-E8-IR	PLCVLEQLYWGESLFVYCSG	8.0	1.2	4.3	0.3	3.5
F820-4-H3-IR	HLCLLEELFWGEALWGYCSG	17.5	5.6	9.0	0.3	3.4

		Ratios over Background E-Tag IGFSR II	Backgrou IGFsR	nd IR		Comparisons IGFR/IR IR/IGFR
Sequence HLCVLEELFWGASLFGYCSG	FGYCSG		[]	1 (; 0
QLCVMEELFWGASRFGQCSG	RFGQCSG	6.4		7		
HLCVLEELFWGASMFGQCSG	4FGQCSG	n a) · ·	3.6	0.	4
QLCVLEEMFWGGSRFVQCSA	FVQCSA), r. r.	1.2	3.2	0.4	
PLCILEELFWGEALFDQCGA	JFDQCGA	25.55	2.4	6.1	0.4	
YLCVQEELFWGASLFGYCSV	LFG1CSV	15.9	1.6	4.1	0.4	
HLCALEEAFFGPSLFNSCQG		8.99	1.9	4.7	0.4	
HLCVLEERFWGASLFGQCSG	LFGQC8G		0.8	1.9	0.4	
QLCDLEELFWGASLFG1CFG	LFGYCFG	22.2	3.1	7.0	0.4	
HLCVLEERFWGASIWGSCSG	IMGSCSG	1. 4	1.1	2.4	0.5	
QLCVLEELFWGGSLWGQCSK	LWGQCSK	 	6.0	1.9	0.5	
PLCVLEELFWGAAQFGQCSG	Predese	4	1.3	2.5	0.5	
QLCDLEERFWGVSLFGLCSG	LFGLCSG	13.0	1.1	2.1	0.5	
QLCVLEEVFWGASLFGLCTG	LFGLCTG	5.01 4 OL	1.2	2.0	9.0	
QL. DLNTWSGLCLCSVTVRV	CSVTVRV	7.2	2.2	3.4	9.0	
DLCVLEESLWGKALFGYCSD	JFGYCSD	9.0	2.5	2.8	6.0	
HLCVLEEVFWGSSMFGDCSG	4FGDCSG		5.6	2.9	6.0	
HLCDLEELFWGASLFGDCQG	JFGDCQG		2.3	2.1	1.1	
QLCVLDALMWGGCRLGHQCG	RLGHQCG) .	1.6	1.5	1.1	
QLCVLEEKFWGTSLFGDCMG	LFGDCMG	0.4.C	9.0	5.0	1.2	
HLCVLEEVFWGAAQFGSCSG	OFGSCSG	•	3.2	2.5	1.3	
OLCVLEELFWGPSMFGYCSG	IFGYCSG); , (C	0.4	2.3	1.8	
HLCDLEELFWGASGFAQCYG	FAQCYG	6:17	•			

Figure 4D (Con't)

		Ratios ove	Ratios over Background	pun	Comparisons	isons
Clone	Sequence	E-Tag	IGFSR	¥	IGFR/IR	IR/IGFR
Design	HLCVLEELFWGASLFGYCSG	1	<i>i</i>	;	;	1 1
A6L-3-C4-IR	DLCVLEERFWGASLFGQCSG	36.9	1.0	40.5	<0.1	42.5
A6L-3-D7-IR	QLCVLEELHWGASLFGYCSG	38.6	1.0	40.1	<0.1	40.7
A6L-3-A1-IR	PLCVLEEQFWGASLFGQCSG	39.6	1.1	44.8	<0.1	40.6
A6L-3-C1-IR	YLCDLEERFWGASLFGQCSS	37.3	1.0	40.3	<0.1	40.3
A6L-3-D5-IR	HLCLLEERFWGSSQFGFCSG	42.9	1.1	44.4	<0.1	40.2
A6L-3-A4-IR	HLCVLEELFWGASQFGQCSG	26.7	1.1	42.2	<0.1	40.2
A6L-3-D3-IR	HLCYLEERFWGASLFGQCSG	34.6	6.0	36.9	<0.1	.39.8
A6L-3-B1-IR	HLCVMEELFWGTSLFGQCTG	33.9	1.0	38.7	<0.1	39.3
A6L-3-B5-IR	HLCVLEERFWGASLFGQCSG	35.3	1.1	42.4	<0.1	38.6
A6L-3-B2-IR	HLCVLEERFWGASLFSQCSG	38.1	1.1	42.7	<0.1	37.7
B6H-4-G12-IR	HLCVLEELFWGASLFGQCSG	31.6	1.1	39.6	<0.1	36.7
B6C-4-H10-IR	QLCLLEELFWGAASFGQCSG	38.5	1.1	41.1	<0.1	36.5
B6H-4-G8-IR	HLCVLEEMFWGASLFGQCSG	31.7	1.1	39.7	<0.1	36.2
A6L-3-D6-IR	HLCDLEELFWGASLFSQCSR	35.5	1.0	37.2	<0.1	36.1
B6C-4-F1-IR	QLCVLEELFWGASQFGYCSG	32.9	1.1	38.7	<0.1	35.8
B6C-4-H3-IR	QLCALEEQFWGASLFSQCSG	37.4	1.2	40.5	<0.1	34.8
B6H-4-E8-IR	QLCVLEELFWGASLFGYCSG	30.2	1.0	35.7	<0.1	34.3
B6C-4-G1-IR	HLCVLEEWFWGDSLFGQCSR	34.9	1.2	40.2	<0.1	33.7
B6H-4-E9-IR	HLCVLEERFWGASLFGQCSG	34.4	1.2	38.8	<0.1	33.2
B6C-4-F5-IR	QLCELEEVFWGASLFDYCSG	34.7	1.2	39.6	<0.1	32.8
B6C-4-F11-IR	HLCVLEELFWGASRFGQCSG	34.0	1.2	37.2	<0.1	31.7
B6C-4-E6-IR	HLCVLEELFWGASLFGQCSA	32.3	1.2	37.4	<0.1	30.6
B6C-4-E12-IR	HLCVLEELIWGASRFGQCSG	30.9	1.1	33.3	<0.1	30.2
B6C-4-G10-IR	HLCVLEELFWGGSLFIQCSG	33.0	1.3	40.3	<0.1	30.1
B6C-4-F8-IR	QLCVLEEQFWGASLFGNCSG	36.4	1.4	39.8	<0.1	29.3
20C-3-B5-IR	HLCVLEERFWGAALFGQCSG	26.6	1.1	32.5	<0.1	29.2
B6C-4-G3-IR	HLCILEEMFWGASLFGQCGG	34.0	1.4	38.8	<0.1	28.3
20C-3-B7-IR	PLCVLBELVWGASLFVQCSG	29.5	1.2	32.9	<0.1	28.3

Figure 4E

		Ratios ove	Ratios over Background	pun	Comparisons	risons
Clone	Sequence	E-Tag	IGFSR	꼼	IGFR/IR	IR/IGFR
Design	HLCVLEELFWGASLFGYCSG) 	;	1	1	1
20C-3-B4-IR	NLCVLEELFWGESLFGQCSG	28.9	1.1	31.1	<0.1	28.0
20C-3-C11-IR	HLCVLEEQFWGGSLFGYCSR	30.2	1.1	31.0	<0.1	27.7
B6C-4-G2-IR	HLCFLEEVFWGAALFAQCSG	29.4	1.3	35.3	<0.1	27.5
20C-3-B8-IR	HLCDLEVLFWGSALFGQCSG	28.5	1.1	31.2	<0.1	27.4
20C-3-C10-IR	HLCVMEELFWGASLFGQCSG	32.1	1.2	33.6	<0.1	27.1
20C-3-B6-IR	HLCVLEERFWGASLFWQCSG	29.7	1.2	31.9	<0.1	26.7
A6L-3-A3-IR	HLCVLEEQYWGESLFGYCSG	14.4	1.1	28.3	<0.1	26.5
A6L-3-B3-IR	PLCVLEEQFWGASLFAYCSS	38.7	1.7	43.4	<0.1	26.3
20C-3-A5-IR	QLCVLEELFWGESLFAQCLG	22.9	1.1	27.6	<0.1	26.0
20C-3-B11-IR	HLCVLEELFWGQSLFGHCSD	30.0	1.3	32.7	<0.1	25.8
20C-3-B3-IR	HLCVLEELVWGASLFGFCSG	29.3	1.2	31.2	<0.1	25.7
20C-3-C12-IR	LLCVLEEQFWGASLFGQCSG	29.6	1.3	31.8	<0.1	24.8
20C-3-C3-IR	RLCVLEELFWGESLFGQCSG	30.1	1.2	30.1	<0.1	24.3
20C-3-C2-IR	HLCVLEEMFWGASLFGNCSG	29.9	1.3	29.8	<0.1	23.8
20C-3-A11-IR	ELCFLEELFWGASLFGQCSG	25.9	1.2	27.4	<0.1	23.0
20C-3-A4-IR	HLCVLEELFWGASLYGQCSS	27.2	1.2	27.5	<0.1	22.9
20C-3-A6-IR	HLCVLEELFWGASLFAQCPG	26.1	1.2	27.5	<0.1	22.8
B6C-4-E4-IR	NLCVLEELFWGASEFGQCSG	34.5	1.7	39.1	<0.1	22.7
20C-3-A9-IR	DLCVLEEQLWGASLFRYCSG	29.7	1.3	29.3	<0.1	22.7
B6C-3-C5-IR	HLCVLEEQFWGVALFGNCSG	33.5	1.7	37.7	<0.1	22.5
20C-3-B1-IR	HLCVLEVQIWGASLFGQCSG	30.2	1.2	26.7	<0.1	22.0
20C-3-A10-IR	HLCVLEERFWGGALFGQCTA	29.0	1.3	28.5	<0.1	21.5
20C-4-F1-IR	HLCDLEELFWGASLFGQCSG	29.1	1.4	29.5	<0.1	20.7
20C-4-E1-IR	OLCVLEELFWGTSLFAGCSG	28.3	1.4	29.7	<0.1	20.6
20C-3-B12-IR	QLCGLEELFWGASLFGYCSA	27.0	1.3	25.8	<0.1	20.2
20C-3-A8-IR	HLCVLEELFWGASIFGQCSS	21.1	1.1	21.2	0.1	20.0
20C-3-A7-IR	FLCVLEELYWGASQFGQCSG	21.9	1.3	23.0	0.1	18.3
B6C-4-E10-IR	HLCVLEEQFWGASLFGYCSG	35.2	2.2	38.0	0.1	17.5

Figure 4E (Con't)

Clone Design	Sequence HLCVLBELFWGASLFGYCSG RT.CALEELFWGASLFGQCSG	Ratios ove E-Tag	Ratios over Background E-Tag IGFsR IR 21.0 1.1 17	9.	Comparisons IGFR/IR IR/IGFR	isons IR/IGFR 16.6
20C-3-C1-IR A6L-3-D2-IR B6C-4-G12-IR B6H-4-F9-IR B6C-4-E3-IR	HLCVLEELFWGAALFHQCSG RLCVLEEQFWGASLFGQCSG QLCVLEELFWGSSRLGYCSG DLCVLEELFWGASLFGQCSG QLCLLEEQFWGGSLFGQCSG HLCVLEELFWGTSLFGQCSG	30.6 7.0 31.1 39.3 34.6 29.9	1.1 2.5 3.6 5.3 16.9	14.9 33.5 43.1 40.0 31.7 25.3	00.000000000000000000000000000000000000	14.1 13.6 12.1 7.6 1.9
20C-3-A3-IR	RLCV LEELVWGAS LF DZCSK					

		Ratios ov	Ratios over Background		Comparisons	sons
Clone	Sequence	E-Tag	IGFsR	IR 24.2	GFR/IR R/ GFR 	R/IGFR 17.3
Parental/Design	WIDDEWAWVOCEVIGRECPS	48.0	1.0		<0.1	48.4
D815-4-A8-1K	WIDDEWAQVQCEVIGAGCES WIDDEWAQVQCEVIGAGCES	49.2	1.0	48.2	<0.1	48.2
-4-DIU-4-	WEDQEWAY CEVI ONCOL	٠	1.0	48.0	<0.1	48.0
D815-4-D9-IR	WID VERWARVOOR VIOLEN STATE OF THE STATE OF	47.9	1.0	48.0	<0.1	48.0
-4-M11-	W.EDEBMING & CENTRAL MAINTONE OF THE MANAGEMENT	49.0	1.0	47.6	<0.1	47.6
D016-4-512-18	WI FORWADVOCEVYGRGCPS	45.4	1.0	47.2	<0.1	47.2
7 - 7	WIDEEWEWIOCKVYGRGCPA	49.5	1.0	47.0	<0.1	47.0
7 7	WIEDEWAWVOCEVYGRGCOS	48.1	1.0	46.6	<0.1	46.6
1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	WINDEWAWIOCEVYGRGCPA	47.8	1.0	46.4	<0.1	46.4
വ	GI,DWEWAWI,OCEVYGRGCPS	47.7	1.0	45.8	<0.1	45.8
10013-4-70-11 10016-4-150-17	WIEDEWEOVRCLVYGRGCPP	47.8	1.0	45.8	<0.1	45.8
D813-4-E7-1K	WINDEWAWOCEVYGRGCPY	49.0	1.0	45.6	<0.1	45.6
D813-4-B10-110	WI.DORWAGVI.CEVYGRGCPS	49.0	1.0	45.6	<0.1	45.6
DOIS-4-RO-IK	OT DESCRIPTION OF THE PROPERTY	47.0	1.0	45.6	<0.1	45.6
D813-4-510-11	MI BORWACHYYCHS CROSS	44.5	1.0	45.4	<0.1	45.4
Dals-4-Clark	MI PPFWAOVOCAVYGRGCSS	44.2	1.0	44.2	<0.1	44.2
Dolora - Gorifa	WI.DOFWAI.VOCEVYGRGCPS	44.3	1.0	43.7	<0.1	43.7
n L	MIDNEMALINGCETT COOKED	45.5	1.0	43.0	<0.1	43.0
D815-4-E11-1K	WILD CEMPAYOFFY GROOTS WITHOUTH WAS CREATED AND A CONTROL OF CONTR	46.2	1.0	43.0	<0.1	43.0
D815-4-h/-ik		47.2	1.0	42.6	<0.1	42.6
D815-4-F12-1R	WIDGERSWINDOW TOTAL OF THE WILDOWN TO THE WILDOW TO THE WI	47.9	1.0	42.6	<0.1	42.6
D815-4-E9-IR	OLDOEWAWULCKVYGRGCPS	46.4	1.0	41.8	<0.1	41.8
D815-4-A10-TR	WI.DHE*AWVOCEVYGRGCPS	47.3	1.0	41.2	<0.1	•
D815-4-C7-TR	OLEOFWAWVRCEVYGRGCSS	37.7	1.0	40.0	<0.1	⁻.
	WI.DORWAWVOCOVYGRGCI.S	47.0	1.0	39.8	<0.1	39.8
ו כוני	WIDDEWAWVRCRVYGLGCPS	44.2	1.0	39.8	<0.1	39.8
7 - 4 - 5 7 - 4 - 5 10 - 4 - 5	WI.DORWAVMKCELYGRGCPS	40.4	1.0	39.5	<0.1	39.2
, ,	MIEDEWAWVOCEVYGRGCLS	45.4	1.0	38.6	<0.1	38.6
D015 4-1112-110	STUDEWAWVOCEVYGRGCLS	37.3	1.0	37.3	<0.1	37.3
D813-4-A/-IN	WI.DHEWAWVOCRVYGRGCTS	2.4	1.0	37.2	<0.1	37.2
D815-4-F7-IR	WLDVEWAWVQCEVYGRGCPS	32.4	1.0	34.7	<0.1	34.7

Figure 6B

		Katios ov	Katios over Background	nna	Comparisons	SOUS
Clone	Sequence	E-Tag	IGFSR IR	¥	IGFR/IR IR/IGFR	VIGFR
Darontal /Design	WINDEWAWOORVYGRECPS	1 1	1	1	1	1
Parencar/Design	OLDOFWARVRCEVWGRGCSS	27.8	1.0	33.6	<0.1	33.6
01 CO 7 100	MIDIEMADVOCKVYGRGCPS	34.7	1.0		<0.1	32.3
D015-4-G/-IK	MIDERWAWOCOVYGRGCPS	30.7	1.0	28.6	<0.1	28.6
D015-4-GII-IN	MIDORWAWYOCEVWGRAPE	33.0	1.0		<0.1	26.4
DOID=4-E/-IK	M. DORONOTHVY GRAND ST. IN STREET ST. IN ST. IN STREET ST.	28.4	1.0		0.1	19.0
D815-4-A12-1K	MIDAFWEWVOOFVYGRGCRD	22.1	1.0	18.8	0.1	18.8
D815-4-D8-IR	SLDREWAYVQCQVYGRGCSS	20.8	1.0	14.6	0.1	14.6
	ı					

		Ratios ov	Ratios over Background	pun	Comparisons	isons
Clone	Sequence	E-Tag	IGFSR	R	IGFR/IR IR/IGFR	R/IGFR
Parental/Design	WLDQEWAWVQCEVYGRGCPS	44.8	1.4	24.2	0.1	17.2
D820-3-H2-IR	RLDLEWANIQCEVYGRGCPS	23.9	1.0	40.0	<0.1	40.0
D820-3-C4-IR	WLEQEWARVQCEVYGRGCSS	31.0	1.0	39.5	<0.1	39.5
D820-3-C3-IR	WLEQEWILVECEVYGRGCPT	35.2	1.0	39.4	<0.1	39.4
D820-3-G6-IR	WLEQEWAQVQCEVWGRGCPS	33.8	1.0	38.8	<0.1	38.8
D820-3-D2-IR	WLDQEWEWIQCEVYGRGCPL	35.6	1.0	37.8	<0.1	37.8
D820-3-D3-IR	LLDEEWAQIECEIYGRGCPS	34.8	1.0	37.7	<0.1	37.7
D820-3-B5-IR	ALEEEWAWVQCEVYGRGCHF	34.1	1.0	37.1	<0.1	37.1
D820-3-E2-IR	C?EQEWGLVQCEVYGRGCPS	34.4	1.0	37.0	<0.1	37.0
D820-3-B3-IR	WLEQEWAYVQCEVYGRGCPS	33.6	1.0	36.7	<0.1	36.7
D820-3-B6-IR	WLEHEWAQVQCEVWGRGCPY	31.2	1.0	36.6	<0.1	36.6
D820-3-D4-IR	WLEQEWAEVRCEVYGRGCPR	32.0	1.0	36.2	<0.1	36.2
D820-3-C2-IR	?LEQEWAWVQCEVYGRGCPS	33.7	1.0	35.6	<0.1	35.6
D820-3-F6-IR	WLEQEWAGIQCKVYGRGCPS	30.8	1.0	35.2	<0.1	35.2
D820-3-D5-IR	RLEQEWAQVQCEVWGRGCLP	30.5	1.0	34.8	<0.1	34.8
D820-3-F5-IR	QLDHEWAGIQCEVWGRGCPS	29.8	1.0	34.6	<0.1	34.6
D820-3-H3-IR	WLEQEWAQIQCEVYGAGCRS	30.2	1.0	33.8	<0.1	33.8
D820-3-G2-IR	SLEQEWAWVQCVVYGRGCPI	31.3	1.0	33.0	<0.1	33.0
D820-3-H6-IR	WLEQEWDQVLCEVYGRGCPY	30.3	1.0	32.2	<0.1	32.2
D820-3-F3-IR	WLEQEWAQV?CEVYGRGCA?	28.6	1.0	30.7	<0.1	30.7
D820-3-B4-IR	WMDQEWAWVQCEVYGRGCPS	33.1	1.0	30.5	<0.1	30.5
D820-3-C5-IR	QLDQEWAWIQCEVYGRNCRT	29.1	1.0	30.3	<0.1	30.3
D820-3-F4-IR	TLEQEWAQVICEVYGRGCLS	25.9	1.0	29.5	<0.1	29.5
D820-3-H5-IR	RLEQEWAQVQCEVWGRGCLS	26.3	1.0	28.6	<0.1	28.6
D820-3-A6-IR	WLDQEWALVQCEVYGRGCPA	24.8	1.0	26.0	<0.1	26.0
D820-3-A2-IR	WLDQEWAQIQCHVWGRGCPA	23.7	1.0	25.6	<0.1	25.6
D820-3-G5-IR	WLEQEWAWVQCEVYGRGCPS	22.6	1.0	25.0	<0.1	25.0
D820-3-G3-IR	RLEEEWAWVQCQVYGRGCPS	22.2	1.0	23.9	<0.1	23.9
D820-3-E3-IR	WLEQEWVRIQCEVYGRGCPS	20.6	1.0	22.7	<0.1	22.7

		Ratios ove	Ratios over Background	pun.	Comparisons	Sons
Clone	Sequence with the second section of the second seco	E-Tag 44.8	IGFSK 1.4	1K 24.2		17.2
Parental/Design	WILD FORWTWOOF VGCCPS	25.9	1.0	22.6	<0.1	22.6
D820-3-E3-IR	MELKENAGVOCETYGRGCPS	27.3	1.0	22.4	<0.1	22.4
D820-3-DI-IR	WIEFEWAWVRCEVYGRGCOS	22.4	1.0	21.9	<0.1	21.9
D820-3-E1-1K	WI PHEWAD TO CELLY CRECTY	22.0	1.0	21.0	<0.1	21.0
D820-3-F1-IA	A LEBENDA WOOFEVYGROOPS	13.1	1.0	18.4	0.1	18.4
D820-3-B2-IA	MI BOBWAOWOFVYCROTORS	23.5	1.0	18.4	0.1	18.4
D820-3-A3-1K	WIENEWAY CONTROL S	25.6	1.0	17.5	0.1	17.5
D820-3-H4-1K		14.5	1.0	16.3	0.1	16.3
D820-3-GI-IR	Characam I WOODWOOD OO	27.8	1.0	13.9	0.1	13.9
D820-3-C1-1R	WEEQEWEEVQCGVIGNOCES	14.7	1.0	12.8	0.1	12.8
D820-3-A1-1K	WEDZEWANIĘCE V IGNOCINO	6.4	1.0	6.3	0.2	6.3
D820-3-A5-IK	WDEXCEMPS(VCEVSCRIP)	13.7	1.0	6.3	0.2	6.2
D820-3-HI-IK	M:DQEMADIQCEVIGRACES	0.9	1.0	4.3	0.2	4.3
D820-3-A4-IR	SLUEEWAGVLCEVIGROCFF	9.9	0.6	10.9	0.8	1.2
D820-4-E12-IR	SVDQELEWLMCHFQGRVCF3				9	0
D820-4-B12-IR	WLEQERAWIWCEIQGSGCRA	32.2	о Ю	1.0	0	•

Figure 6C (Con't)

		Ratios over	Ratios over Background	pun	Comparisons	sons
Clone	Sequence	E-Tag	IGFsR	۱R	IGFR/IR I	IR/IGFR
Parental/Design	WLDQEWAWVQCEVYGRGCPS	44.8	1.4	24.2	0.1	17.3
D820-3-D5-IGFR	WVNQALGGVQSDVQGRRCQS	29.6	3.8	1.0	3.8	0.3
D820-3-E4-IGFR	LLDHEWPWVGCEVCGRGSLS	27.1	3.2	1.0	3.2	0.3
D820-3-C5-IGFR	WLHQELAWVRGEGYPRGRRS	25.0	3.1	1.0	3.1	0.3
D820-3-F4-IGFR	WLGHDWAWIQCEVYGLGCPC	3.9	2.7	1.0	2.7	0.4
D820-3-F6-IGFR	WIDQEGVRVQCEA*GRAFPS	26.7	2.6	1.0	2.6	0.4
D820-3-G4-IGFR	WRDEEWAWVQGVVQGRGWPA	3.8	5.6	1.0	2.6	0.4
D820-3-E2-IGFR	RLGVEWSWFQRKVYGRDSTS	15.3	2.6	1.0	2.6	0.4
D820-3-G6-IGFR	WLAQGWAGVQCVVYGRGCRN	20.3	2.4	1.0	2.4	0.4
D820-4-E11-IGFR	WLEEE*AGIQCQV?GRGCPS	12.6	1.0	3.0	0.3	3.0
D820-4-H11-IGFR	WLDQEWEWVQCEVWGRGCLS	8.1	1.0	4.6	0.2	4.6
D820-4-D11-IGFR	RLEQEWALIQCEVYGRGCPS	4.5	1.0	5.3	0.2	5.3
D820-4-A8-IGFR	WLEEEWAQVQCQVYGRGCAS	3.2	1.0	5.5	0.2	5.5
D820-4-F9-IGFR	WLDLE*EWLQCEVYGRGCAT	9.4	1.0	5.8	0.2	5.8
D820-4-C8-IGFR	WLEQEWVQVRCEVYGRGCPS	11.6	1.0	5.9	0.2	5.9
D820-4-D9-IGFR	WLEEEWAQVQCEVYGRGCPS	10.1	1.0	8.9	0.1	8.9
D820-4-D7-IGFR	WLDQEWARVQCEVWGRGCTY	34.1	3.5	33.4	0.1	9.5
D820-4-H9-IGFR	YLD?EWAWVQCEVYGLGCQS	18.4	1.0	10.1	0.1	10.1
D820-4-E10-IGFR	WLDVE*AWVQCEVWGRGCPS	26.7	2.6	27.0	0.1	10.4
D820-4-E7-IGFR	WLEQEWER?QCEVYGRGCPP	31.9	3.0	32.2	0.1	10.7
D820-4-H8-IGFR	WLEEEWAQVQCEVYGRGCLS	16.1	1.0	11.7	0.1	11.7
D820-4-A11-IGFR	WLDQEWAWIQCEVYGRGCPS	8.0	1.0	12.5	0.1	12.5
D820-4-C9-IGFR	?LEHEWAQIQCEV?GRGCQS	19.6	1.0	14.9	0.1	14.9
D820-4-E9-IGFR	WL?QEWAWIQCEVYGRGCPF	19.3	1.0	17.3	0.1	17.3
D820-4-B10-IGFR	WLD?EWAWVQCEVYGRGCPS	19.3	1.0	21.5	<0.1	21.5
D820-4-F10-IGFR	GLEQGCPWVGLEVQCRGCPS	27.8	1.0	25.7	<0.1	25.7
D820-4-B9-IGFR	WLEEEWAWVQCEVYGHGCPS	31.7	1.0	26.5	<0.1	26.5
D820-4-G8-IGFR	WLDQEWAQIQCEVYGRGCSS	25.6	1.0	29.3	<0.1	29.3

		Ratios ove	Ratios over Background	pun	Comparisons	sons
Clone	Sequence	E-Tag	IGFsR	~	IGFR/IR I	IR/IGFR
Parental/Design	WLDOEWAWVOCEVYGRGCPS	44.8	1.4	24.2	0.1	17.3
D820-4-G9-1GFR	WI,DOEWAOVOCEVWGRGCPS	36.8	1.0	29.6	<0.1	29.6
D820-4-C10-IGER	WLDLEWEFVOCEVYGRGCPT	32.6	1.0	31.3	<0.1	31.3
D820-4-A9-IGFR	WLEOEWASVOCEVYGRGCPS	20.4	1.0	31.4	<0.1	31.4
D820-4-B8-1GFR	WLDLEWEOIKCKVYGRGCPF	31.1	1.0	32.7	<0.1	32.7
D820-4-F8-IGFR	WLEOEWAOIOCOIYGRGCPS	28.3	1.0	32.9	<0.1	32.9
D820-4-H7-IGFR	WLEOEWALVLCEVYGHGCPA	34.1	1.0	32.9	<0.1	32.9
D820-4-E8-IGER	WIEOEWAOIOCEVWGRGCSS	26.6	1.0	33.2	<0.1	33.2
D820-4-G10-IGFR	WLE?EWEWVOCEVYGRGC?S	37.5	1.0	33.2	<0.1	33.2
D820-4-D10-TGFR	WLEOEWAOVOCDVYGRGCPS	36.6	1.0	33.5	<0.1	33.5
D820-4-D8-TGFR	WLEOE*ARVOCEVWGRGCPS	23.7	1.0	34.6	<0.1	34.6
D820-4-A10-IGFR	WL?OEWARVHCEVWGRP?OC	29.4	1.0	35.5	<0.1	35.5
D820-4-B7-IGFR	PLEHEWAWVOCVVYGRGCRS	35.4	1.0	36.9	<0.1	36.9
D820-4-E12-IGFR	SLE? EWAWVOCEV? GRGCP?	37.0	1.0	37.0	<0.1	37.0
D820-4-H10-IGFR	WLDOEWVRVOCEVWGRGCPS	36.8	1.0	37.1	<0.1	37.1
D820-4-F12-TGFR	SLDKEWAWVKCEVYGRGCPS	36.9	1.0	37.3	<0.1	37.3
D820-4-F7-IGFR	LGDOEWAWVEWEV#GRGWPS	34.4	1.0	37.5	<0.1	37.5
D820-4-G12-IGFR	WLEEEWAOIRCGVYGRGCPS	30.3	1.0	37.8	<0.1	37.8
D820-4-D12-IGFR	WLEEE*GWVOCEVWGRGCPP	37.2	1.0	38.6	<0.1	38.6
D820-4-A12-IGFR	CLDOEWA?VOCPVYGRGCPS	30.4	1.0	39.3	<0.1	39.3
D820-4-C12-IGFR	OLELEWARVOCEVWDRGCPS	37.1	1.0	39.6	<0.1	39.6
D820-4-A7-IGFR	RLEOEWAWIOCEVYGRGCRF	35.4	1.0	40.8	<0.1	40.8
D820-4-B12-IGFR	SLEHE*AWVQCKVYGRGC?S	36.2	1.0	41.4	<0.1	41.4

		Ratios ove	er Backgro	pun	Comparisons	sons
Clone	Sequence	E-Tag	E-Tag IGFSR IR		IGFR/IR	
	IR/IGFR					
Parental/Design	WLDQEWAWVQCEVYGRGCPS	44.8	1.4	24.5	<0.1	17.3
B6-4-G12-IR	WLDQEWAWIQCEVYGRGCPP	4.4	1.0	6.9	4.4 1.0 6.9 0.1 7.1	7.1
B6-3-A11-IR	WLDQEWAQVRCEVYGRGCPS	7.3	1.0	6.3	0.2	6.3

	Sequence	HIR affinity mol/I
J228	HPPLEHLKAFLL-NH2	2.4*10 ⁻⁵
J229	APTFYAWFNQQT-NH ₂	2.4*10 ⁻⁶
S122	HPTSKEIYAKLLK	9.3*10-6
S123	HPSTNQMLMKLFK	1.6*10 ⁻⁵
S124	HPPLSELKLFLIKK	2.3*10 ⁻⁵

Figure 7

J105	ACVWPTYWNCG Ac-CVWPTYWNCG Bz-CVWPTYWNCG Ac-ACVWPTYWNCG ACVWPTYWACG	5.0*10 ⁻⁶ 3.0*10 ⁻⁵ 3.2*10 ⁻⁵ 4.5*10 ⁻⁵
J104 J105	Bz-CVWPTYWNCG Ac-ACVWPTYWNCG	3.2*10 ⁻⁵
J105	Ac-ACVWPTYWNCG	
		4.5*10 ⁻⁵
	ACVWPTYWACG	
J109		2.0*10 ⁻⁵
J110	ACVWPTYANCG	2.4*10 ⁻⁵
J111	ACVWPTAWNCG	3.1*10 ⁻⁵
J112	ACVWPAYWNCG	3.3*10 ⁻⁵
J113	ACVWATYWNCG	5.5*10 ⁻⁵
J115	ACAWPTYWNCG	2.7*10 ⁻⁶
J116	AAVWPTYWNAG	3.4*10-5
J117	ASVWPTYWNSG	2.9*10 ⁻⁵
J118	ACPYNWVTWCG	2.9*10 ⁻⁵
J119	ACVWPTYWnCG	3.2*10 ⁻⁵
J120	ACVWPTYWNCG	3.4*10 ⁻⁵
J121	ACVWPTyWNCG	1.8*10 ⁻⁵
J122	ACVWPtYWNCG	5.1*10 ⁻⁵
J123	ACVWpTYWNCG	2.5*10 ⁻⁵
J124	ACVWPTYWNCG	2.0*10 ⁻⁵
J125	ACVWPTYWNCG	1.8*10 ⁻⁵
J127	acvwptywncg	4.4*10 ⁻⁵
J128	gcnwytpwvca	5.3*10 ⁻⁵
J130	AEVWPTYWN(Dpr)G	1.9*10 ⁻⁵
J131	ACDWPTYWNCG	5.5*10 ⁻⁵
J132	AC(Leu)WPTYWNCG	4.5*10 ⁻⁶
J133	AC(dLeu)WPTYWNCG	2.8*10 ⁻⁵
J134	AC(IIe)WPTYWNCG	7.4*10 ⁻⁶
J135	AC(dlle)WPTYWNCG	2.9*10 ⁻⁵
J136	AC(Met)WPTYWNCG	7.5*10 ⁻⁶

FIGURE 8

J137	AC(dMet)WPTYWNCG	2.5*10 ⁻⁵
J138	AC(Abu)WPTYWNCG	7.8*10 ⁻⁵
J139	AC(dAbu)WPTYWNCG	2.1*10 ⁻⁵
J140	AC(Nva)WPTYWNCG	3.6*10 ⁻⁶
J141	AC(dNva)WPTYWNCG	3.0*10 ⁻⁵
J142	AC(tBuG)WPTYWNCG	3.2*10 ⁻⁵
J143	AC(dtBuG)WPTYWNCG	3.8*10 ⁻⁵
J144	AC(Phe)WPTYWNCG	5.1*10 ⁻⁶
J145	AC(dPhe)WPTYWNCG	5.7*10 ⁻⁵
J146	AC(Cha)WPTYWNCG	2.2°10 ⁻⁵
J147	AC(dCha)WPTYWNCG	1.7*10 ⁻⁵
J148	AC(NaI(1))WPTYWNCG	5.8*10 ⁻⁶
J149	AC(dNal(1))WPTYWNCG	2.0*10 ⁻⁵
J150	AC(Acy)WPTYWNCG	2.0*10 ⁻⁵
J151	ACVWPT(Hyp)WNCG	2.2*10-4
J154	ACVWPT(Nal2)WNCG	8.2*10 ⁻⁵
J155	ACVWPT(MetO2)WNCG	1.9*10 ⁻⁴
J157	ACVWPT(Cha)WNCG	1.2*10 ⁻⁴
J160	ACVWPT(Ser)WNCG	1.8*10-4
J162	ACVWPT(Thi)WNCG	2.5*10 ⁻⁴
J163	ACVWPT(dSer)WNCG	5.0*10 ⁻⁵
J166	ACVWPT(dCha)WNCG	7.5*10 ⁻⁵
J170	ACVWPT(dPhe)WNCG	1.4*10 ⁻⁴
J171	ACVWPT(Thr)WNCG	7.7*10-4
J174	ACVWPT(Phe)WNCG	4.5*10 ⁻⁵
J176	ACVWPT(dThr)WNCG	2.8*10-5
J180	ACVWPTYW D CG	5.6*10 ⁻⁵
J182	ACVWPT D WNCG	2.7*10 ⁻⁵
J183	ACVWP D YWNCG	3.3*10 ⁻⁵
J184	ACVW D TYWNCG	6.2*10 ⁻⁵

Figure 8 (Con't)

J185	ACV D PTYWNCG	3.4*10 ⁻⁵
J186	AC D WPTYWNCG	3.5*10 ⁻⁵
J187	ACVWTYWNPCG	4.3*10 ⁻⁵
J188	ACVWTYWPNCG	3.0*10 ⁻⁵
J189	ACVWTYPWNCG	3.1*10 ⁻⁵
J190	ACVWTPYWNCG	2.6*10 ⁻⁵
J191	ACVPWTYWNCG	3.0*10 ⁻⁵
J192	ACPVWTYWNCG	4.2*10 ⁻⁵
J193	ACWPTYWNVCG	4.8*10 ⁻⁵
J194	ACPTYWNVWCG	4.2*10 ⁻⁵
J195	ACTYWNVWPCG	3.3*10 ⁻⁵
J196	ACYWNVWPTCG	2.4*10 ⁻⁵
J197	ACWNVWPTYCG	2.9*10 ⁻⁵
J198	ACNVWPTYWCG	4.2*10 ⁻⁵ -
J199	ACVWPCG	4.7*10 ⁻⁵
J200	CVWPTYWNCG	5.5*10 ⁻⁵
J201	ACWWPTYWNCG	6.8 * 10 ⁻⁶
J202	ACEWPTYWNCG	4.6*10 ⁻⁶
J203	ACRWPTYWNCG	5.8*10 ⁻⁶
J204	ACQWPTYWNCG	9.2*10 ⁻⁶
J205	ACGWPTYWNCG	4.4*10 ⁻⁶
J207	cyclo-Valeroyi-AWPTYWNCG	5.5*10 ⁻⁵
J208	cyclo-Toluyl- AWPTYWNCG	7.6*10 ⁻⁵
J209	cyclo-Acetyl- AWPTYWNCG	7.7*10 ⁻⁵
J210	(WPTYWNCG) ₂	5.3*10 ⁻⁵
J211	(AWPTYWNCG) ₂	7.9*10 ⁻⁶
J212	ACA(Bpa)PTYWNCGK(biotin	1.8*10 ⁻⁵
J213	ACAWPTY(Bpa)NCGK(biotin	1.8*10 ⁻⁵
J214	GCAWPTYWNCG	1.4*10 ⁻⁶
J215	NCAWPTYWNCG	9.0*10 ⁻⁶

Figure 8 (Con't)

J216 VCAWPTYWNCG J227 SFYEAIHQLLGV-NH2 J228 HPPLEHLKAFLL-NH2 J229 APTFYAWFNQQT-NH2 S122 HPTSKEIYAKLLK S123 HPSTNQMLMKLFK S124 HPPLSELKLFLIKK	
J228 HPPLEHLKAFLL-NH2 J229 APTFYAWFNQQT-NH2 S122 HPTSKEIYAKLLK S123 HPSTNQMLMKLFK	2.8*10 ⁻⁶
J229 APTFYAWFNQQT-NH ₂ S122 HPTSKEIYAKLLK S123 HPSTNQMLMKLFK	6.4*10 ⁻⁶
S122 HPTSKEIYAKLLK S123 HPSTNQMLMKLFK	2.4*10 ⁻⁵
S123 HPSTNQMLMKLFK	2.4*10 ⁻⁶
	9.3*10 ⁻⁶
C424 LIDDI CEL VI EL IVV	1.6*10 ⁻⁵
S124 HPPLSELKLFLIKK	2.3*10-5

		Ratios ove	Ratios over Background	pun	Comparisons	risons
Clone	Sequence	E-Tag	IGFsR	띰	IGFR/IR IR/IGFR	IR/IGFR
H5 Parental	LCOSLGVTYPGWLAGWCA	:	1.2	:	!	1
H5-3-JBA5-IGFR	LCQSWGVRIGWLAGLCP	31.9	16.3	!	!	1
H5-3-E1A11-IGFR	VCOSLGITDLGLCAGWGA	21.3	8.0	!	; 1	1
H5-3-E4B10-IGFR	LCOSLGLTHPGFEAWLCA	29.7	7.8	1	1	1
H5-3-E4C10-IGFR	LCONFGVTDPGCFYGWFA	24.3	6.1	1	t I	!
H5-3-JBB6-IGFR	PCORLGDTHLCWLAGWFA	40.2	5.4	:	; 1	; 1
H5-3-E4A9-IGFR	LCOSSGLSFLGCLGWWA	27.7	4.3	;	1	!
H5-3-E2A12-IGFR	LCOSLGFTDLDWLACWFE	27.2	4.2	;	1	1
H5-3-E4A12-IGFR	VCOGLGVECPGWFAGWWA	27.9	3.9	!	1	1 1
H5-3-E1F9-IGFR	PCOSLGLTCSGWFEGWGA	18.6	3.5	!	•	:
H5-3-E4F11-IGFR	LCOGWGIRIGWLVGRCM	28.4	3.3	1	1	:
H5-3-E4A11-IGFR	LWOSVGIKYPGGLAGWLA	31.0	3.0	!	1	:
H5-3-E4G7-IGFR	OWOSIGVICPGSWAELCA	26.2	2.2	:	1	:
H5-3-E1B9-IGFR	LCQSLGVTYWEGLAWLCA	20.0	2.1	1	1	1

		Ratios ov	Ratios over Background	punc	Comparisons	sons
Clone	Sequence	E-Tag 31.5	IGFsR 20.6	₹ 1.0	IGFR/IR IK/IGFR 20.6 <0.1	K/IGFR <0.1
JBAS Parental	I COCMOUNT TOWN TOWN TOWN	46.8	41.5	1.0	41.5	<0.1
JBAS-4-2CIZ-IGEN	TOPOWOVE TOWN ACT.	48.1	39.5	1.0	39.5	<0.1
TENE DELETED TOER		42.5	39.5	1.1	35.9	<0.1
UBAS-Z-IEIO-IGIN		44.1	40.2	1.2	33.5	<0.1
JBAS-4-ZAII-IGFN		34.7	33.3	1.0	33.3	<0.1
UBAS-3-2AS-1GFR		34.6	33.1	1.0	33.1	<0.1
UBA5-4-2A9-1GFR		39.6	31.4	1.0	31.4	<0.1
UBA5-1-1B0-1GFK	TOOMDATACASECT	39.6	22.3	1.0	22.3	<0.1
UBAS-4-2BS-1GFR		24.9	22.6	1.2	18.8	0.1
UBAS-1-10FA	# # # # # # # # # # # # # # # # # # #	35.5	15.3	1.1	13.9	0.1
1545-3-2C5-1GFR		26.2	14.8	1.5	9.9	0.1
1545-1-16. C 146F		39.4	4.5	1.0	4.5	0.2
JBA5-2-1D12-IGFR	L*KSWDVRSGLMAGLCP	42.2	2.2	1.0	2.2	0.5

		Ratios ove	Ratios over Background		Comparisons	sons
Clone	Sequence	E-Tag	IGFSR IR		IGFR/IR IR/IGFR	IR/IGFR
Design	LCQSWGVRIGWLAGLCP	1	:	1	:	1
JBA5-4-G12-IR	LCQSWDACIQWLVGLSP	37.5	3.0	1.4	2.1	0.5
JBA5-4-G3-IR	LCRSWEECIGWLVGPQP	4.5	2.5	1.1	2.3	0.4
JBA5-4-G1-IR	LCQSWGECIDRLVGQGA	32.0	3.2	1.3	2.5	0.4
JBA5-3-B1-IR	LCQCWGVRIGWLAGLCP	29.4	6.8	1.2	5.7	0.2
JBA5-3-C1-IR	LCOGWAVHIGOLAGLCP	36.3	7.5	1.1	6.8	0.1
JBA5-3-A6-IR	LCQGWGVHIGRLAGLCP	28.0	7.4	0.7	10.6	0.1
JBA5-3-A2-IR	LCQSWGVRIGWLAGLCP	10.2	4.8	0.4	12.0.	0.1
JBA5-3-B7-IR	LCOSWGVHIGRLAGLCP	39.2	15.2	1.2	12.7	0.1

Figure 9C

		Katios ov	Katios over Background		Comparisons
Clone	Sequence	E-Tag	IGFSR	R	E-Tag IGFSR IR IGFR/IR IR/IGFR
	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX	!	;	1	1 1
nearan		a 7 c	37 B 7 E	ŀ	1 1
R20-4-C10-IGFR	PKGTRFRGDVDVWDGYSWLA))) •		

		Ratios ove	Ratios over Background	pun	Comparisons	isons
Clone	Sequence	E-Tag	IGFSR	¥	IGFKIK IKIGFK	KIGFK
Dealon	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX	1	; !	l I	1	: !
	S.IWTYSWGGYTWI,S	10.9	3.7	0.5	7.3	0.1
20f-4-B/-1GfR	S. TWENCOLLETTING TO BE SEEN TO SEE THE SEED OF THE SE	6.8	4.7	0.7	6.3	0.2
20F-4-E4-1GFR	S. TWHY SOMMING OF COMMING	7.6	4.7	0.8	6.0	0.2
20F-4-E12-1GFR	STRUCTURE CANNOT AND A CANNOT A C	13.9	10.1	1.8	5.6	0.2
20F-4-F4-IGFR		13.7	3.9	0.8	5.1	0.2
20F-4-F/-1GFR	SDVWAQPQNRNDWFGIIIMBS	7.2	2.5	0.5	4.7	0.2
20F-4-E7-1GFR	KFHKINFQUDAVWFGIDMEG	7 7 6	16.2	W.	4.6	0.2
20F-4-F11-IGFR	HRGTVTGVWVAKWFGIEWDS		; -		1 4	0
20F-4-D10-IGFR	FGRGYGGDGGGYWSGYEWLA	ν	7) ·		
20F-4-B3-IGFR	DGLVVKSGREWPGYGWLER.A	17.3	14.4	3.6	4.0	7.0
205-4-D12-TGED	DGSTV_VSSSVGWPGYEWLM	10.1	6.6	2.4	4.0	0.5
ZOF-4-BIZ-IGIN	W.TWCYSDWASSINA. TNACOW	9.9	2.7	0.7	4.0	0.5
20F-3-A9-1GFR		5.1	1.3	0.5	2.7	0.4
20F-4-62-1GFR		2.0	1.0	0.5	2.3	0.4
20F-4-D11-1GFR		6. E	6.0	0.5	1.8	9.0
20F-4-G4-1GFR 20F-4-G12-IGFR	MGGGLWVGVIII NEGIONEDS SDVWAQPQRRNDWPGYHWLS	3.2	6.0	9.0	1.5	0.7

		Ratios ov	er Backgro	nnd	Compar	ISOHS
3	Cocnonco	E-Tag	IGFSR	R	E-Tag IGFSR IR IGFR/IR IR/IGFR	IR/IGFR
Clone	achaeta company and a company)	1	,	1	- 1
Dogian	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX	1	:	1		
The state of the s		C C	۲,	٥ 4	٠,	8.0
JOE 4 U10 TD	T.D.L.A.G.D.S.W.I.G.Y.D.VI.R.G.W.L.S	7.01	٠,) - i	
VIT - 0 TU - 4 - 107		7	0	4	۲	0.4
20F-4-C10-IR	IHSSDGIGAWGGYAWFRDVA	#· c 2))	1	•	

Figure 10B (Con't)

		Ratios ove	er Backgro	nuq	Comparisons	SOUS
5		E-Tag	E-Tag IGFSR IR I	IR I	GFR/IR	IR/IGFR
Clone	XXXXXXXXXXXXXXXXX	1 +	1	1	1	! !
Design		21.5	1.0	8.0	0.1	8.0
R2015-4-D10-1K		32.6	6.8	15.1	0.5	32.6 6.8 15.1 0.5 2.2
R20 -4-D9b-1R		11.6	1.7	3.6	0.5	2.1
R20 3-4-H4-IR		1 7 1	9 0	5, 2	0.5	2.0
R20 3-4-A2-IR	GRVALWGPVWPRWWFMSRPV	7.7	3	1		

Ratios over Background Comparisons	E-Tag IGFSR IR IGFR/IR IR/IGFR		44.6 1.5 2.7 0.6 1.8	0 0)
	Sequence	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX		KGT.KT.DKLMKSGGFALVFRWFCFSIRCEVEMLINIGSFS	GRISMAFVPPRHLQPELAPRPVRNHAWLVGGG
	Close		Design	R40-3-40A2-IR	R40-4-40F10-IR

į	c	Ratios ov	Ratios over Background	^	Comparisons	sons B/ICFR
Cione	Sequence	10 - 1	:		;	;
200-3-H3-1GPP	DHRICGTDEYLMODLFVRGLCRLIW	28.5	26.6	1.0	26.6	<0.1
20C 3 113 IGEN	GLI.PCKOLFTI.AGI.OPEAGCVSSSR	34.4	27.5	1.2	23.1	<0.1
20C 3 11 1CIN	TWIACLDELLRGOVWSSCRRAPIG	35.5	24.4	1.3	19.2	0.1
20C-3-G5-TGFR	DWI,RCI,GVILSGGLTELANTGCVOG	29.3	21.1	1.1	18.7	0.1
20C-3-A2-TGFR	WFSFCI.GGI.LOAOEWSVWGRDVGCI	33.9	18.3	1.1	16.9	0.1
20C 3 112 IGFR	GYSWLRDVLMEKOAOLKREGSVGRO	39.8	29.1	1.9	15.2	0.1
20C-3-C6-TGFR	FI.TRI.LERI.GLS*ERGEAGGPYAOA	34.8	20.9	1.4	14.9	0.1
200 0 CO TOTA	PSGFCMGLERLSOVSLGYCGAGOGG	34.8	28.1	2.0	14.2	0.1
20C 3 EZ IGFR	1 S F R COL, F V L, A GM H P C P V D V G G E G F	33.7	14.3	1.2	12.4	0.1
20C J 13 ICIN	NTPNCSODWGOESGFMALLLALTCK	30.2	9.8	6.0	11.2	0.1
20C 3 EX ICEN	TGOYG(17), ATTUTATUTATUTATUTATUTATUTATUTATUTATUTAT	35.5	31.9	3.9	8.2	0.1
20C-3-F3-IGFR	GESTONILARAOTVELAL/TEMQVOE	33.3	19.3	2.8	6.9	0.1
20C-4-A7-1GFR 20C-4-F8-TGFR	T.S.P.A.T.I.SOT.SGVVI,PDCI.I.GED	30.5	27.7	5.3	5.2	0.2
20C-4-G11-TGFR	GEHFCOLL, MSI, CGDDCGPVNCGGGS	24.7	13.3	2.8	4.7	0.2
20C-2-F1-TGFF	GWFFCT.T.AST.VI.OVPOGRSRASAVC	34.0	5.1	1.6	3.1	0.3
20C-3-B6-IGFR	YRQECACSVGAVGFLCGLACLARSG	37.3	32.8	13.7	2.4	0.4

		Ratios ov	Aatios over Background	pun	Comparisons	sons
Clone	Sequence	E-Tag	IGFSR IR	IR	IGFR/IR IR/IGFR	R/IGFR
Design	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX	1	:	1	:	1
40F-4-D1-IGFR	LSCLAYSRHGIWRPSTDLGLGRSVGEGSVSTRWRGYDWFE	4.9	4.6	0.3	13.1	0.1
40F-4-B1-IGFR	GLDHSDAVGVHLGFAWPA. ARGRWEAGGLEDTWAGYDWL	4.1	3.0	0.2	13.1	0.1
40F-4-D10-IGFR	W.GYAWLS	4.9	4.5	0.4	11.7	0.1
40F-3-A3-IGFR	LSCLAYSRHGIWRPSTDLGLGRSVGEGSVSTRWRGYDWFE	2.6	2.0	0.3	7.9	0.1
40F-4-C4-IGFR	EAMAVGLQCPARFVRAAAHGDGGSWGQDHV.AWGGYWWLG	3.8	2.0	0.5	4.1	0.2

Figure 10F (Con't)

Ratios over Background Comparisons	IGFSR IR	39.1 1.8 27.7 0.1 15.4	7.9 1.0	1.0 2.0	1.0 2.0	7 0 1 0 1 0 5
	Seguence	HLCVLEELFWGASLFGYCSG	HFYVI,VERLSGASLFGSGSA	HRFVREGLLMGAYOFCYCSG	FOSTITEET, VWGA PLERYGTG	
	Clone	Parental/Design	F815-4-G11-TGFR	F815-3-D1-IGER	F815-4-C10.TGFR	

		Ratios ove	Ratios over Background	pun	Comparisons	sons
Clone	Sequence	E-Tag	IGFSR	IR	IGFR/IR I	IR/IGFR
Parental/Design	HLSVLEELSWGASLFGQWAG	5.4	1.0	2.1	0.5	2.1
NNKH-4-A9-IR	NLCRLEELAWGASLFGQCAG	16.3	1.0	2.7	0.4	2.6
NNKH-4-H4-IR	APVSTEELRWGALLFGQWAG	15.6	1.0	5.6	0.4	2.5
NNKH-4-B3-IR	HLSVLEERWWRESLFGQWAG	13.6	2.8	6.7	0.4	2.3
NNKH-4-E1-IR	HLSVLEERWWRAALFGQWAG	13.9	4.8	9.5	0.5	2.0
NNKH-4-E7-IR	HLSILEEQWWRESLFGQWAG	16.9	1.3	2.3	9.0	1.8
NNKH-4-G3-IR	HMSVEELSWWASLFGKQAG	11.3	1.3	2.3	9.0	1.7
NNKH-4-B6-IR	HLSELEERWWRATLFGQWAG	13.2	1.3	2.1	9.0	1.7
NNKH-4-A10-IR	HLSVLEELWWRESLFGQWAG	15.4	2.0	3.2	9.0	1.6
NNKH-4-A5-IR	HLSLLEEQWWRESLFGQWAG	14.6	4.6	6.9	0.7	1.5
NNKH-4-F11-IR	HLSVLEERWWRETLFGQWAG	14.0	3.1	3.9	0.8	1.3
NNKH-4-C9-IR	HLSVLEEQWWRESLFGQWAG	14.3	2.3	2.9	0.8	1.3
NNKH-4-D12-IR	HLSVLEEQWW.ESLFGQWAG	12.0	1.4	1.7	0.8	1.2
NNKH-4-D10-IR	HLSVLEELWWREALFGQWAG	13.6	1.2	1.5	0.8	1.2
NNKH-4-E5-IR	HLSVLEERWWRATLFGEWAG	14.5	1.4	1.6	6.0	1.1
NNKH-2-A6-IR	HL.VLEELLWGVSLFRQWAG	8.4	1.4	1.5	1.0	1.1
NNKH-4-F6-IR	HLSALEEQWWRATLFGQWAG	14.1	2.8	2.9	1.0	1.0
NNKH-4-C7-IR	HLSVLEERWWRATLLESGQ	14.7	1.4	1.4	1.0	1.0
NNKH-4-F7-IR	HLSALEELWWRETLFGQWAG	14.1	7.5	7.0	1.1	6.0
NNKH-4-F8-IR	HLSVLEELWWRESLFGKWAG	13.6	11.0	8.6	1.3	0.8
NNKH-4-E9-IR	HLSVLEEAWWRESLFGHWAG	15.5	7.9	6.0	1.3	0.8
NNKH-4-E6-IR	HMSEQEELWWRATLFGQWAG	18.2	3.8	2.7	1.4	0.7
NNKH-4-B7-IR	HLSVLEERWWRETLFGEWAG	16.5	12.9	8.2	1.7	9.0
NNKH-2-B3-IR	HRSVLKQLSWGASLFGQWAG	11.5	5.3	0.7	7.4	0.1

		Ratios ove	Ratios over Background		Comparisons	sons
Clone Parental/Design	Sequence H.SVI.EELSWGASLFGOWAG	E-Tag 5.4	IGFSR 1.0	IR 2.1	IGFR/IR IR/IGFR 0.5 2.1	R/IGFR 2.1
NNKH-2-C5-IGFR	HL*VLEELSWGASLVGQWAV	7.3	6.0	0.7	1.3	0.8
NNKH-2-D9-IGFR	HLSVLEEL*LGASMFGLWAG	4.1	0.5	0.4	1.3	0.8
NNKH-2-H12-IGFR	HLSVLKELSW*ASLFGQWAG	5.0	1.3	1.1	1.2	8.0
NNKH-2-D10-IGFR	HLSALEELSWGASLFGQWAG	4.8	2.1	1.9	1.1	6.0
NNKH-2-G9-IGFR	HLSVLAELS*GALLFGQWAG	1.9	1.4	1.3	1.1	6.0
NNKH-2-C6-IGFR	RLSVLEQLSWGASLFGPWAG	18.2	1.0	6.0	1.1	6.0
NNKH-2-C7-IGFR	HL*VLVQPSWGASLFGQWAG	21.8	1.3	1.3	1.0	1.0
NNKH-2-F11-IGFR	HQSVLEELSR*ASLFGQWAG	6.7	1.3	1.4	6.0	1.1
NNKH-2-H3-IGFR	DMSVLGGLSWGA*LFGQWSG	4.7	0.7	0.8	6.0	1.1
NNKH-2-B8-IGFR	HLSVREGOLWRASMFGRWAG	17.5	3.7	5.2	0.7	1.4
NNKH-2-B12-IGFR	OLSVLVEL*WGASLFGPWAA	1.2	1.0	2.9	0.3	2.9
NNKH-2-F9-IGFR	HLSVGEELSW*VALLGQWAR	3.7	9.0	2.1	0.3	3.5

	Clonal	Formula	K, (µM)		Fat Cell		K, (µM)	Ratio	
D Name	Name	-32	HIR	P0,	Assay	Activity	HIGFR	IGF/IR	Sequence
D101	20D3		0 51 0 27				13 11	25	KIGGQGQHQDGNFYDWFVEALAKK (£-biotin)
D102	20D1	_	12 0 97				7.4	6.2 16	KVLQARHGCDSVSDCFYEWFAKΚ (ε-biotin)
D103	B8	_	0.74				15	20	KWSALLSVMDTGFYAWFDDAVKK (e-biotin)
DIO	E7		20			-	>20	~	KGHSWALVRHVDRLFYEWFDLKK (E-biotin)
D105	811	_	2.8				12	43	KRDKPTDQEEQNWSFYEWFRHKK (c-biotin)
D106	20F1	_	0 97				6.2	6.4	KVFWNCRSQQLDFYEWWFEQAAKK (ε-biotin)
D107	40G11	-	1.1	YES		Antagonist	6.7	8.8	KLESHYVVPQAALDRLFYSWFSKK (e-biotin)
D108	3G11	1	2.3			Antagonist	61	8.3	KFYGWFSRQLSLTPRDDWGLPKK (ε-biotin)
D109	20111		36			Antagonist	12	3.3	KSAPGLVSNKQDGLFYSWFREKK (ε-biotin)
D110	G3	-	0.84			Antagonist	1.4	1.7	KRGGGTFYEWFESALRKHGAGKK (c-biotin)
D111	102	_	0.62				3.2	5.2	KDPERMQSDVGFYEWFRAAVGKK (e-biotin)
D112	1GFR C1 A65-4-C1	_	0.49			Neutral	0.05	0.1	DYKDCWARPCGDAANFYDWFVQQASKK (e-biotin)
	IGFR 112								
D113	A65-4-1+2	_	0.75		~20 µM	Agonist	5.4	7.2	DYKDVTFTSAVFHENFYDWFVRQVSKK (E-biotin)
D114	IGFR A6	_	8.1			Neutral	>20	>2.5	SAKNFYDWFVKK (e-biotin)
D115	IGFR D5	_	8.1				>20	>2.5	ADKNFYDWFMAAKK (e-biotin)
D116	IGFR JBA5	6	4.4 cycli		>20 µM	Agonist	8.1	1.8	DYKDLCQSWGVRIGWLAGLCPKK (e-biotin)
D117	IGFR 112C	-	0.70	YES	~20 µМ	Agonist	6.1 5.1	8 6 8.5	F! IENFYDWFVRQVSKK(e-biotin)
D118	20E2	2	0.25	YES	~20 µM	Agonist	1.3	5.2	DYKDFYDAIDQLVRGSARAGGTRDKK (e-biotin)
D119	20C11	2	0.25	YES	~20 µM	Agonist	13 2.5	2.9 0.8	KDRAFYNGLRDLVGAVYGAWDKK (ε-biotin)
D120	E8	9	0.37			Antagonist	2.2	5.9	KVRGFQGGTVWPGYEWLRNAAKK (c-biotin)
D121	F2	01				Antagonist	7.4	6.7	KSMFVAGSDRWPGYGVLADWLKK (ε-biotin)
D122	20A4 (A7)	9	1.2			Antagonist	>20 >20	>17 >20	KEIEAEWGRVRCLVYGRCVGGKK (ε-biotin)
D123	D8	9	0.55			Antagonist	16 >20	29 >15	KWLDQEWAWVQCEVYGRGCPSKK (e-biotin)
D124	F8	4	0.04				8.2 >20	200 >200	KHLCVLEELFWGASLFGYCSGKK (e-biotin)
D125	IGFR E4	_	26				>20	>8	DYKDERSAAGFRGNFYDWFVAQVNKK (e-biotin)
D126	IGFR D2C	_	14				18	13	LGENFYDWFVMQVRKK

Figure 11A

Clonal	D or S			IR-Kd	IR-ICs	IR-IC, FP.	PO4	Fat Cell
Name	name	Motif	Sequence		Blacore	21.0		ASSRY
20-F2	10118	98	DYKIDFYDAIDQLVRGSARAGGTRDK K-biotin	250 nM		2.8 nM	+	‡
CI	1)112	94	DYKIXWARPCGDAANFYDWFVQQAS KK-biotin	490 nM			,	0
1)8	1)123	C-C LOOP	KWI.DQI;WAWVQCEVYGRGCPSKK	550 nM			0	•
E8	10120	GROUP 6	KRGFQGGTVWPGYEWLRNA	370 nM			•	•
- œ	1)124	C-C 100P	KHILCVLEELFWGASLFGYCSGKK	40 nM			•	0
112C	7110	9V	FHENFYDWFVROQVSKK	700 nM	>5 µM	S nM	‡	‡
KCF9			RLYYEWFWGQLEAQGRGGLS					
KC-G2		2-2-3	GLEQGCPWVGLEVQCRGCPS					
KCG7		B6	FYCGLEELSWGAALFGYCSG		<1 µm			
NG-C2		136	GNGIXGMFYQLLSLLVGRDMH					
NG-G33		A6	GIISQSCPESFYDWFAGQVSDPWWCW		2-4 µM	4.2 nM	‡	
NG-G8		B6	VEGRGLFYDLLRQLLARRQNG		>5 µM		•	
NG-G9		B6	RAMSFYDALVSVLGLGPKK-Biotin				•	
RP-1		A6	GSRPVF1IEQFYEWFVDQLGL		I puM		+	
RP-2		A6	RSEASFIIVIEFYSWFEEQLRS		l μΜ		+	
RP-3		۷6	GRFYGWFQDAIDQLMPWGFD		>10 µM		- 4	
RP4		B6	PPWGARFYDAIEQLVFDNI.		S puM		+	
RP-5		136	AGVNAGFYRYFSTLI,DWWDQGKK-Biotin		₩п 9		,	
RP-6		B6 +	TFYSCLASLIGTPQPNRGPWERCRKK-Biotin				‡	
7 00		AA	AAVIIFOFYDWFADOVKK					
RP-8		136	OSFYDYIEELLGGEWKK		×5 µM		+	
RP-8#	\$287	136	OSFYDYTEELL.GGEWEE					
RP-9		A6	GSLDESFYDWFERQLGKK			2.9 nM	‡	
RP-10		136	GSFYEALQRLVGGEQGKK		>10 µM		+	
RP-11		A6	QAPSNFYDWFVREWDKK		>10 µM		+	
RP-12		B6	DPFYQGLWEWLRESGKK					
RP-13		A6	ASGFPENFYDWFGRQLSLKK		>10 µM			
RP-14		A6	SACQFDCIENFYDWFARQKK		>10 µМ			
RP-15		A6	SQAGSAFYAWFDQVLRTVKK					
RP-16		B6	V,DARDD[FUJL;SE;VTLL					
RP-17		B6	QSDAFYSGLWALIGLSDGKK		>10 µM			
RP-18		B6	LQPCSGFYECIERLIGVKK					
RP-19		A6	LKDGFYDYFWQRLHLGSKK					
RP-20		B6	GSASFYDAIDRLLRMRIKK					
RP-24		GROUP 6	WPGYLFFEEALQDWRGSTED					
S167	S167	A6	AFYDWFAKK	>20 µM	No Binding		•	
S173	\$173	RB6	LDALDRLMRYFEERPSL	1.2 µM				.
S174	S174	RB6	PLAEI,WAYFEIISEQGRSSAH	16 µМ				
S175	S175	A6	GRVDWLQRNANFYDWFVAELG	230 nM	24 µM	0.9 nM	‡	
8176	S176	A6	NGVERAGTGDNFYDWFVAQLH	470 nM				

Figure 11B

	D or S.			IR-Kd		IR-IC, FP.	PO.	Fat Cell
	name		Sequence		Biacore	SI7S	•	Assay
2177	<i>11</i> 18	98	EHWNTVDPFYFTLFEWLRESG	2.7 µM				+
	S178		EHWNTVDPFYQYFSELLRESG	130 nM				0
	6L1S		QSDSGTVHDRFYGWFRDTWAS	540 nM				‡
	S224		FQSLLEELVWGAPLFRYGTG					
	S225	C-C I,OOP	PLCVL.EELFWGASL.FGYCSG					
	8226		QLEEEWAGVQCEVYGRECPS					
	S264		IQGWEPFYGWFDIDVVAQMFEE					
	S257	196	RWPNFYGYFESLLTIFFS					
	S258	B6	HYNAFYEYFQVI.LAETW					
	8259	B6	EGWDFYSYFSGLLASVT					
						The second secon		

Figure 11B (Con't)

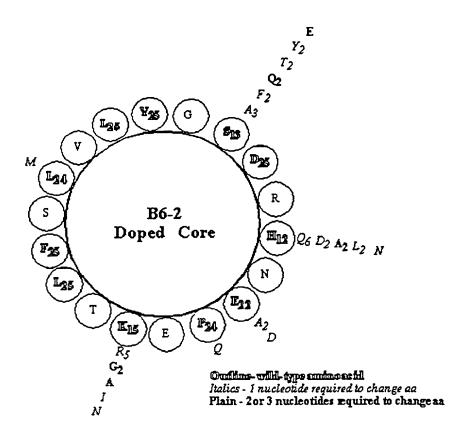


FIGURE 12

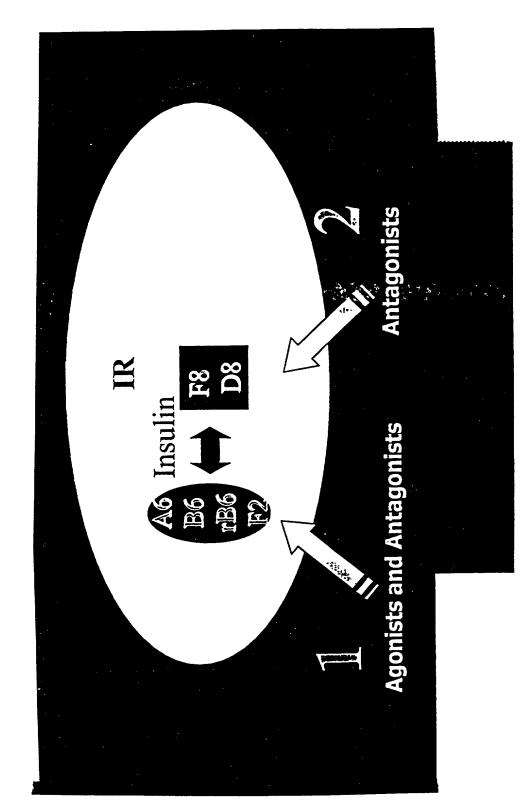


FIGURE 13

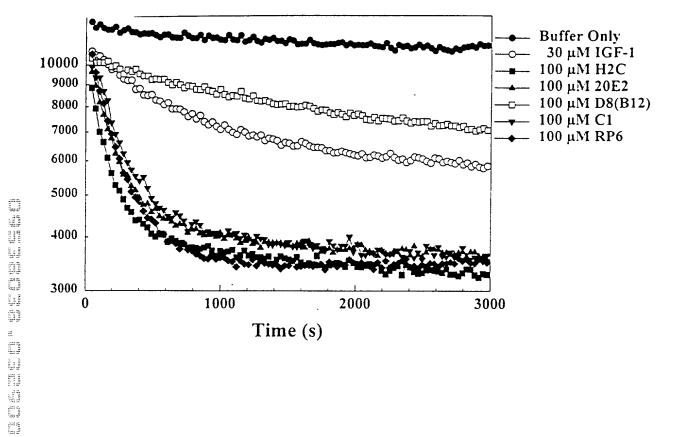
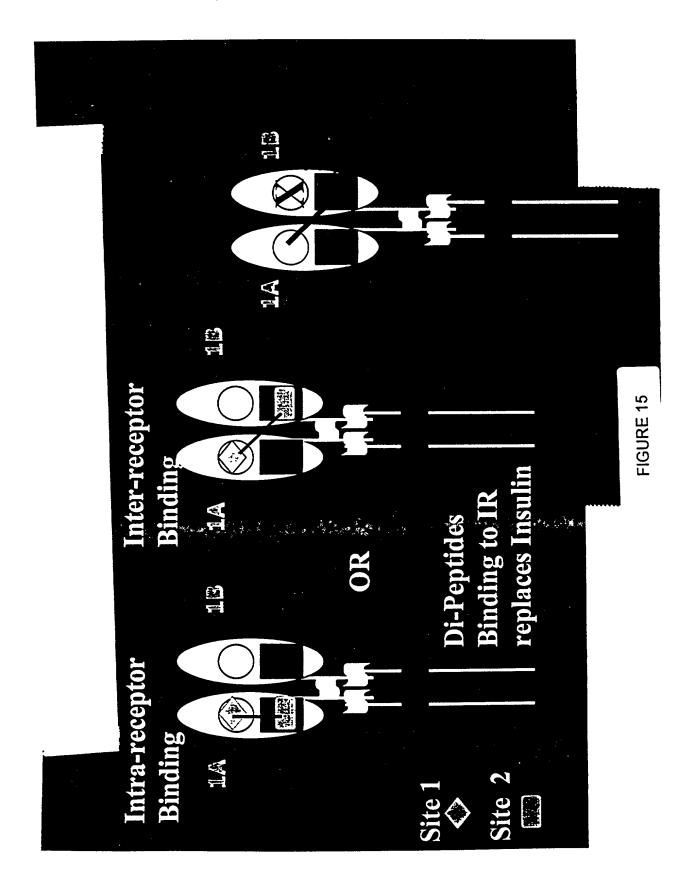
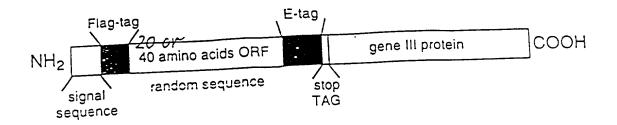


FIGURE 14





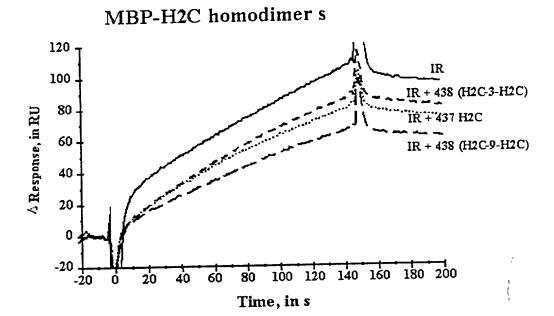


FIGURE 17

Class I clones	# # F	# Clones	Compet-	
B6 3x DYKDAETPAQVGWNRLWSVWPGEHWNTVDPFYHKLSELLRESGA E5 2x DYKDRHLTNAELGVQSPEVLSRLFPDGDIFYRALSHLVRGMGPP B5 2x DYKDRGGMDRQWLDVGARHRLERRSVQDNTDDFYGGLRILVDGF 9 DYKDGPPDSFDVTEKGDMAILNVRFDPHSLDFNDQTFYFLLDSL G6 DYKDAPLDARLSAPRFQWSPRTWRQSLSYGEWSCGSFYDCLEALAASPPT 12 DYKDAPLDARLSAPRFQWSPRTWRQSLSYGEWSCGSFYDCLESI A5 DYKDMGSSQFQDTRPSSGQAYSHSLDSDGWGTANWIFLRALEGL C6 DYKDSGAAHEGNQGRERSTHLAANINDHLPGDAGIWLGYSWLS C0nsensus (regular+frameshifters) A1 CGDRGFYFNKPTGYGSSR A1 CGDRGFYFNKFTGYGSSSR A1 CGDRGFYFNKFTGYGSSSR A2 3 30 A1 CGDRGFYFNKFTGYGSSSR A2 3 30 A3 30 A3 30 A3 A	GA GPP PT SR SA		+ + pu pu +	
Class I frameshifting clones (all in +1 frame) F6 2x TTKTRG.IFGMLLGVLRFQILLWPFPKDCVQMKDIFYSLLASL 7 3x TTKTRIGCCS.LVWGWRGCRLADGFYAFLMALAG 7 TTKTRLLLLLGGDEPFYGLLRMLIGRGS 65 TTKTGWFAWVLAFSVQGVGVAFYSALAALLCAHSASLVCGA	1 SRGS CAHSASLVCGA 1	H 67 H	+ ud nd	
Class II clones D5 DYKDPLYGGGIHLYYPGTMGYVPGFPRQVKVLGDADKNFYDWFM A6 DYKDYRGMLVLGRISDGAGKVASEPPARIGQKVFAVNFYDWFV R35 DYKDSGCCRLLGLRWMFIVIVGWSGALVCQSAASAAGFYDWFV	~ ~ ~		nd	

human IGF mature (1-70) GPETLCGAEL VDALQFVCGD RGFYFNKPTG YGSSSRRAPQ TGIVDECCFR SCDLRRLEMY CAPLKPAKSA

nd = no data

FIGURE 18

139 CACTACAMG ACTCGIGGTT GANTITICGG TAIGITGCTG GGCTTTCAGAT TCTTCTGTGG CCGTTTCTA AGGATTGTAT ICATATTTTT ATTCGTTGTT GGCTAGTTTG GCGGCCGCA DYKDSWINFRYVAGRAQVSDSSVAVS. GLCSDERYFLFVVGQFGGR TTKTRG IFGHLLGVLRFQILLWPFPKDCVQHKDIFYSLLASLAAA LQRLVVEFSVCCMACLGFRFFCGRFFRJFFFKIFFIRGWRRP

8 IGF B
CACTACAAAG ACGCGGTGG GGCGTGGTGGG GGTGATGG GCTTCTCCGT ATGCTGATTG GTGGTGGGCT TGGGGCCGCAA
GACTACAAAG ACGCGGTTGC GGCGTGTTGG GGTGATGG GCTTTTTATGG GCTTCTCGT A D W S W C G R
D Y K D A V A A A V A P W G . . A F L W A S P Y A D W S W V C G R
T T K T R L R L L L L G G D E P F Y G L L R H L I G R G S A A A
T T K T R L R L L L L G G D E P F Y G L L R H L I G R G S A A A
L Q R R G C G C C S L G V H S L F H G F S V C . L V V G L R P ర

FIGURE 19

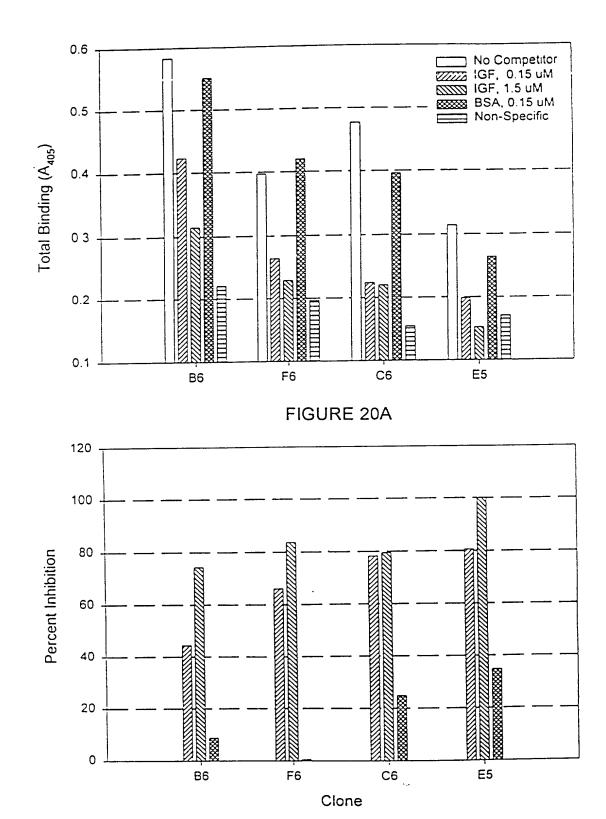


FIGURE 20B

AETPAQVGWNRLWSVWPGEHWNTVDPFYHKLSELLRESGA Peptide 5.1 (18 aa) Clone B6

NTVDPFYHKLSELLREKK (biotin)

QMKDIFYSLLASLAAKK (biotin) MLLGVLRFQILLWPFPKDCVQMKDIFYSLLASL Peptide 5.2 (17 aa) Clone F6

ADKNEYDWEMAAKK (biotin) PLYGGGIHLYYPGTMGYVPGFPRQVK<u>VLG</u>DADK**NFYDWF**M Peptide 5.3 (14 aa) Clone D5

SAKNFYDWFVKK (biotin) YRGMLULGRISDGAGKVASEPPARIGOKVFAVNFYDWEV Peptide 5.4 (12 aa) Clone A6

FIGURE 21

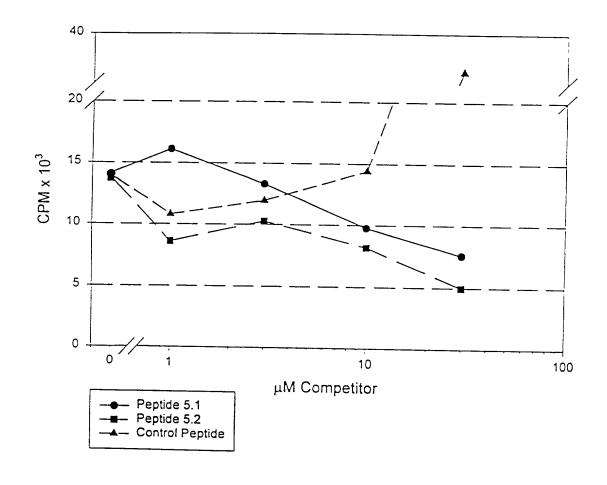


FIGURE 22

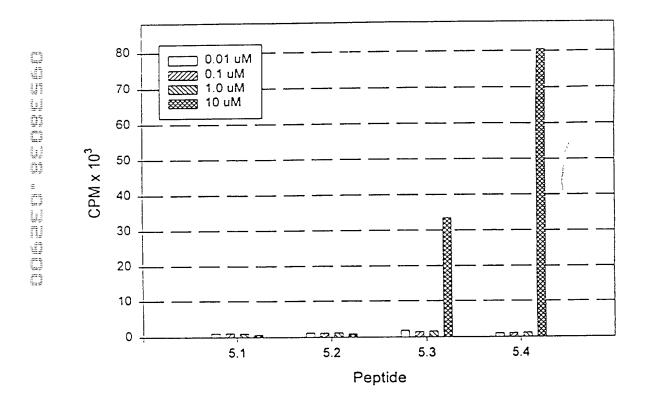


FIGURE 23

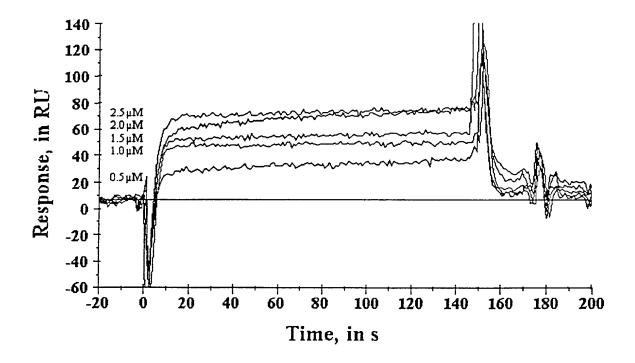


FIGURE 24A



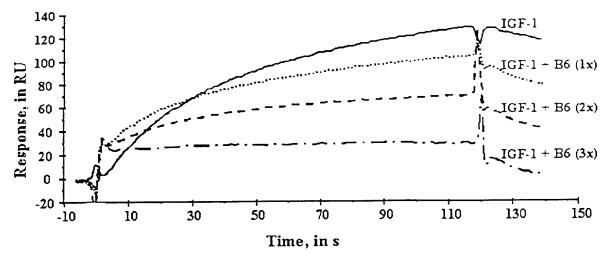


FIGURE 24B

plant, and then the left of the last her being her than the last than the last that the last than the last the l

GACTACAAAGACGACGATGACAAGTACCGTGGTATGCTGGTTCTGGGGTCGTATCTCTGACG
D Y K D D D D K Y R G M L V L G R I S D

GTGCTGGTAAAGTTGCTTCTGAACCGCCGGCTCGTATCGGTCAGAAAGTTTTCGCTGTTAA
G A G K V A S E P P A R I G Q K V F A V N

CTTCTACGACTGGTTCGTTGCGGCCGCA 96 nt
F Y D W F V A A A FIGURE 25A

CTACAAAGACGACGATGACAAGTACCGTGGTATGCTGGTTCTGGGTCGTATCTCTGACGGTGCT
GGTAAAGTTGCTTCTGAACCGCCGGCTCGTATCGGTCAGAAAGTTTTCGCTGTTAACTTCTACG
ACTGGTTCGTTGCGGCCGCAGTGTGA 154 nt

FIGURE 25B

GAC:	IAC.	AAA	GAC	NNK	AAC	TTC	TAC	GAC	TGG	TTCG									
D	Y	K	D	X	X	X	X	X	X	X	X	X	X	N	F	Y	D	W	F

TTNNKNNKNNKNNK 21 aa V X X X X

FIGURE 26A

CTACAAAGACNNKNNKNNKNNKNNKNNKNNKNNKNNKNNKAACTTCTACGACTGGTTCGTTNNK NNKNNKNNKGCGGCCGCAGTGTGA

FIGURE 26B

```
\mathtt{NH_2-D-Y-K-D-L-C-Q-S-L-G-V-T-Y-P-G-W-L-A-G-W-C-A-K-K} \\ \texttt{(Biotin)-COOH} \\ \texttt{NH_2-D-Y-K-D-L-C-Q-S-L-G-V-T-Y-P-G-W-L-A-G-W-C-A-K-K)} \\ \texttt{(Biotin)-COOH} \\ \texttt{(Bioti
 H5
H5 Control NH2-D-Y-K-D-W-C-L-T-L-Q-P-L-V-W-A-S-G-G-Y-C-A-K-K(Blocin)+COOH
                                                                                                       NH2-D-Y-K-D-V-C-Q-R-L-G-G-T-F-P-G-W-L-V-G-V-C-R-K-K(Biotin)-COOH
  2C3-60
                                                                                                       NH2-D-Y-K-D-L-C-Q-R-L-G-V-G-W-P-G-W-L-S-G-W-C-A-K-K(Bictin)-COOH
 H5-447
                                                                                                       NH2-D-Y-K-D-L-C-Q-S-L-G-V-T-W-P-G-W-L-A-G-W-C-A-K-K(Blotin)-COOH
 H5-432
```

Clone:		Binding Ratios: Target E-Tag % M	lax
A6S-1-C5	DYKD RIHNQTERGGNFYDWFVHqLV AAA	7 27 26	3
A6S-1-G3	DYKD VATVHVGGGMNFYDWFVAQVG AAA	5 19 26	3
A6S-1-A2	DYKD KDPVTVSQGRNFYDWFVVqIQ AAA	5 20 25	5
A6S-1-D5	DYKD RVGSGMEDLGNFYDWFVRQAQ AAA	5 25 20	
A6S-1-H4	DYKD HKSWTTMSPLNFYDWFVAQVE AAA	3 18 17	
7,00 1114			
A6S-2-F2	DYKD LAMSVASRPANFYDWFVAqIV AAA	30 35 86	6
A6S-2-D2	DYKD RAERGSMRDSNFYDWFVqQLP AAA	30 36 83	3
A6S-2-E3	DYKD VqEGLSGMEGNFYDWFVDQLF AAA	28 36 78	3
A6S-2-H2	DYKD RGQRESDSGTNFYDWFVGAIR AAA	28 40 70)
A6S-2-A3	DYKD SRAPYGSTAGNFYDWFVqAVS AAA	25 37 68	3
A6S-2-H1	DYKD RVGIqVDPHTNFYDWFVIQLT AAA	27 42 64	\$
A6S-2-F1	DYKD VGqVGRYVRS NFYDWFV QqAM AAA	8 30 27	7
A6S-2-G1	DYKD RPqLVESGSKNFYDWFVqVVR AAA	8 30 27	
A6S-2-B2	DYKD EMYGDTSERVNFYDWFVSALq AAA	5 30 17	
A6S-2-A1	DYKD LSSRGRVTMRNFYDWFVAQVV AAA	3 31 10	
A6S-3-E1(DYKD RVREKLPRPENFYDWFVNqIH AAA	22 23 96	
A6S-3-G2	DYKD TWMWEERKqDNFYDWFVGQLK AAA	20 21 95	
A6S-3-E5	DYKD RYRGERHDGRNFYDWFVEqVN AAA	19 21 90	
A6S-3-H2	DYKD qGAEGRLSEGNFYDWFVQAVS AAA	19 21 90	
A6S-3-H9-	DYKD YSIEVqDWNENFYDWFVSQLG AAA	20 23 87	
A6S-3-G3	DYKD PRLHMGSDMG <u>D</u> FYDWFVVqIA AAA	18 21 86	
A6S-3-F8	DYKD GRGGGLKRPDNFYDWFVAAAK AAA	20 25 80	
A6S-3-G11	DYKD GAVGLAEAGP NFYDWFV SqVq AAA	19 24 79)
A6S-3-H1	DYQD PASNKNSLAE NFYDWFV qQTR AAA	23 30 77	•
A6S-3-E6	DYKD DARDHGVWVMSNFYDWFVAqVS AAA	5 20 25	,
A6S-3-D9	DYKD SLQGADFQQGNFYDWFVSELA AAA	4 17 24	
A6S-3-E3	DYKD RPSLPEVRPGNFYDWFVqSVR AAA	4 19 21	
A6S-3-H8	DYKD NPTSVqQYGVNFYDWFVNVLS AAA	4 20 20)
A6S-3-G4	DYKD CADPGACSSLNFYDWFVgMRG AAA	4 21 19	1
A6S-3-B1(DYKD YDqDPPYWGLNFYDWFVREVA AAA	3 16 19	
A6S-3-C1	DYKD RPVIGGGGTRNFYDWFVAqMI AAA	3 17 18	
A6S-4-G5	DYKD QEVTRTRDDKNFYDWFVSqIF AAA	26 18 14	4
A6S-4-D2	DYKD PPYRSSRLGENFYDWFVMqVR AAA	26 19 14	
A6S-4-F6	DYKD LKGSSQPLSVNFYDWFVQQIK AAA	24 17 14	
A6S-4-H4	DYKD PRMVEKPSEDNFYDWFVTqLS AAA	28 20 14	
A6S-4-C1	DYKD CWARPCGDAANFYDWFVQQAS AAA	22 16 14	
A6S-4-G3	DYKD GAQAIREIHHNFYDWFVAQVT AAA	29 21 13	
A6S-4-H3	DYKD GRGDQRHETTNFYDWFVRELQ AAA	28 20 13	
		· -	

FIGURE 28

A6S-4-H6.	DYKD	GSIAQLIMRANFYDWFVEqTN	AAA	24	18	130
A65-4-G6	DYKD	RLMGGIAEPQNFYDWFVREVA	AAA	25	20	126
A6S-4-H5	DYKD	HHSPGNEHGYNFYDWFVLqVA	AAA	24	19	123
A6S-4-E4.	DYKD	ERSAAGFREGNFYDWFVAqVN	AAA	32	27	120
A6S-4-F5.	DYKD	GSQHSGREPHNFYDWFVAqVG	AAA	28	24	120
A6S-4-D4	DYKD	IARMRETFQPNFYDWFVDQLA	AAA	21	18	118
A6S-4-C6	DYKD	RLDRSSTSGVNFYDWFVAqVG	AAA	28	25	116
A6S-4-D3	DYKD	GLRSEQGNRLNFYDWFVAQIA	AAA	23	20	116
A6S-4-F2	DYKD	SVIQTRQDETNFYDWFV?AMS	AAA	26	23	115
A6S-4-A5	DYKD	VEVQRHIRKDNFYDWFVKQID	AAA	22	19	115
A6S-4-C3	DYKD	VTMLDKGAQDNFYDWFVREVA	AAA	24	21	114
A6S-4-F3	DYKD	HNSSSPMRTGNFYDWFVQELR	AAA	30	26	113
A6S-4-B4	DYKD	ERSPRPALASNFYDWFVQQVV	AAA	21	19	113
A6S-4-B6	DYKD	SDARQAGLQENFYDWFVSQVR	AAA	26	23	113
A6S-4-B1	DYKD	RHERGKEGPGNFYDWFVSQVV	AAA	21	19	112
A6S-4-G4	DYKD	SALSGPVQPINFYDWFVTGM	AAA	30	26	112
A6S-4-A6	DYKD	HVEHMAVGDGNFYDWFVVqLR	AAA	23	21	111
A6S-4-F4	DYKD	VGHSGVPPYPNFYDWFVMQVS	AAA	24	22	110
A6S-4-D6	DYKD	LGAAETWDGINFYDWFVKQVS	AAA	24	22	110
A6S-4-E6	DYKD	RSSGGLLSqGNFYDWFVSQLE	AAA	26	24	109
A6S-4-A3	DYKD	LAINDLVTHKNFYDWFVDQLR	AAA	20	18	109
A6S-4-E3	DYKD	RGMTGMVGRGNFYDWFVGQLR	AAA	23	21	109
A6S-4-A2	DYKD	IGGQGQHQDGNFYDWFVEALA	AAA	22	20	107
A6S-4-B2	DYKD	QSVDLSRPDSNFYDWFVEVLS	AAA	22	21	105
A6S-4-H2	DYKD	VTFTSAVFHENFYDWFVRQVS	AAA	20	19	104
A6S-4-D1	DYKD	SNPSRQDASVNFYDWFVREVA	AAA	22	22	103
A6S-4-H1	DYKD	IVAGARHSEVNFYDWFVIQVR	AAA	18	18	102
`A6S-4-E2	DYKD	?DGQSVSSKGNFYDWFVQqMT	AAA	25	25	101
A6S-4-G1	DYKD	AELVGAGVRGNFYDWFVDQLV	AAA	16	16	101
A6S-4-G2	DHKD	SAGHHMPRES NFYDWFV DQVV	AAA	24	25	99
A6S-4-A1	DYKD	DSSRLWLGERNFYDWFVAqIS	AAA	12	17	68

Name	Sequence	#Found	Ratio	IGF Inh.	GHR*
H5:	LCQSLGVTYPGWLAGWCA	_	1.2	-	2.6
2C3:	VCQRLGGTFPGWLVGVCR	-	1.1	_	1.1
JBA5:	LCQSWGVRI-GWLAGLCP	19	~24.0	~45%	1.2
E2A12:	LCQSLGFTDLDWLACWFE	10	~17.5	~54	1.1
ElAll:	VCQSLGITDLGLCAGWGA	1	16.4	50	1.0
E4B10:	LCQSLGLTHPGFEAWLCA	5	~11.7	~50	1.2
E4C10:	LCONFGVTDPGCFYGWFA	· 1	9.9	~51	0.7
E4A9:	LCQSSGLSFLGCL-GWWA	14	~8.5	~65	1.0
JBB6:	PCQRLGDTHLCWLAGWFA	6	~8.3	~65	1.1
ElF9	PCQSLGLTCSGWFEGWGA	1	8.3	68	1.2
E4G7:	QWQSLGVTCPGSWAELCA	1	6.0	50	1.3
E4A11:	LW Q SV G IKYPGGLAGWLA	1	5.8	67	1.4
E1B9:	LCQSLGVTYWEGLAWLCA	3	5.5	60	1.1
E4A12:	VCQGLGVECPGWFAGWWA	3	~5.3	~55	1.2
E4F11:	LCQGWGIRI-GWLVGRCM	1	2.7	58	1.1
E1D3:	LC Q SL G VTYPGWLAGGCA	1	2.0*	_	1.0

Genomic rvab Library

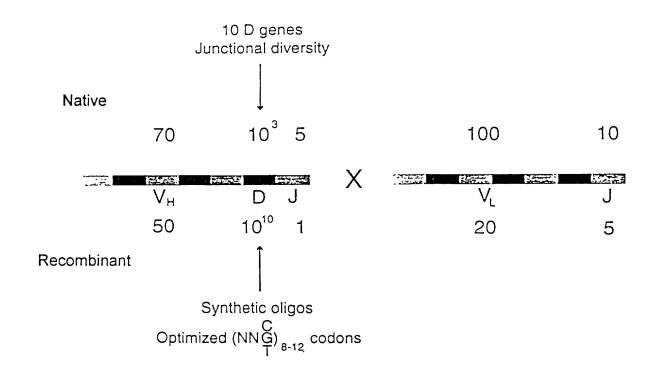


FIGURE 30

VH Gene Sequences	Lambda and Kappa Gene Sequences
DP-1	, , , , , , , , , , , , , , , , , , , ,
DP-10	DPK11
DP-12	DPK15
DP-14	DPK18
DP-15	DPK2/L14+
DP-2	DPK3/L11+
DP-21	DPK4
DP-25	DPK6
DP-29	DPK8/Vd+
DP-30	DPL23
DP-31	HK101
DP-32	L22+
DP-33	L23/L23a
DP-35	LFVK431
DP-38	VA++
DP-39	
DP-40	
DP-42	
DP-44	
DP-45	
DP-46	
DP-47	
DP-5	
DP-50	
DP-51	
DP-52	
DP-53	
DP-54	
DP-59	
DP-63	
DP-66	
DP-67	
DP-68	
DP-69	
DP-7	
DP-70	
DP-71	
DP-73	
DP-74	
DP-8	
hv1263	

VHD26

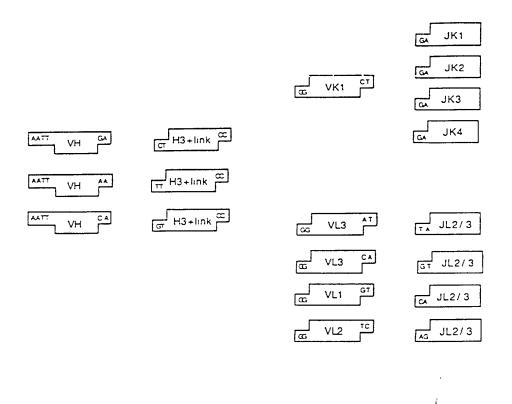


FIGURE 32

The first state of the state of

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CTINIV --- NIVVACCECEGETCCCTTGGGACCAGTGGCAGAGGAGTCCACCTCCGCCAAGTCCGCCTCCACCGAGACCGCCACA
                                                                                                                                                                                                     BOLD = IN DGI COLLECTION
  G4S linker
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         GTGGTATTCGGCGGAGGGACCAAGCTGACCGTCCTAGCGGCCGCAGTGTGAGGTCCAAAAGATTTCG
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               TACACCATAAGCCGCCTCCCTGGTTCGACTGGCAGGATCGCCGGCGTCACACTCAGGTTTTCTAAAGC
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           / Not1 / PCR primer site
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          FIGURE 33
                                                                                                                                                                                                                                                   continued as for J4b gene and linker
                                                                                                                                                                                                                                                                                                                                                                                                                     TGGACGTTCGGCCAAGGACCAAGGTGGAAATCAAAGCGGCCGCAGTGTGAGTCCAAAAGATTTCG
                                                                                                                                                                                                                                                                                                                                                                                                                                             GAACCTGCAAGCCGGTTCCCTGGTTCCACCTTTAG1TTTCGCCGGCGTCACACTCAGGTTTTCTAAAGC
                                                                                                                                                  continued as for J4b gene and linker
                                                                                                                                                                                                                                                                                                                                                                        / Not1 / PCR primer site
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               LeuThrPheGlyGlyGlyThrLysValGlulleLys
CTCACTTTCGGCGGAGGGACCAAGGTGGAGATCAAA continued as for JKl
GAGAGTGAAAGCCGCTCCCTGGTTCCACCTCATTT
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                TyrThrPheGlyGlnGlyThrLysLeuGluIleLys
TACACTTTTGGCCAGGGACCAAGCTGGAGATCAAA continued as for JKl
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         PheThrPheGlyProGlyThrLysValasplleLys
TTCACTTTCGGCCCTGGGACCAAAGTGGATATCAAA continued as for JKl
                                                                                                                                                                                                  DP31, DP33, DP39, DP40, flp1, DP47 and DP49
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 ValValPheGlyGlyGlyThrLysLeuThrValLeu
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        continued as for JL2/3
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        GTG--- continued as for JL2/3
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              continued as for JL2/3
JII4b gene
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        / JL2/3 for DPL16+v3s1+v318
                                                                                                                                                                                                                                                                                                                                                                                               TrpThrPheGlyGlnGlyThrLysValGluIleLys
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  GAATGTGAAAACCGGTCCCCTGGTTCGACCTCTAGTTT
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        GAAAGTGAAAGCCGGGACCCTGGTTTCACCTATAGTTT
                                                                                                                                                                                                                                                                                               3' end for 4 VH genes: DP2, DP3, DP5 and DP38
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                for DPL23+VL3.1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                for DPL2+DPL3
  / CDR 113 /
                                                                                                                                                    NNK - - - NNK
                                                                                                                                                                                                                                                     NNK - - - NNK
                                                                                                                                                                                                                                                                           GTWNM - -- NNM
                                                                                                                                                                          TTNNM - - - NNNT L
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   for DPL11
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 GTCAC---
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              CACAC---
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    AGCAC---
                                                                                                                                                                                                                                                                                                                                                                           JK1 gene
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                JK2 gene
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     JK3 gene
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         JK4 gene
                                                                                                                                                                                                  3' end for 7 VH genes.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                DPL16 (=v3s1)+
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                /DPL23 (=VL3.1)/
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 His
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         NNN-----CAT
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           Ala
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 NNN------GCA
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    NNN-----GGT
  VH gene
                            ۸rg
                                                                                                                                                                                                                                                   CCGGAATTCGGCCCAGCCGGCCNNN - - - - NCA
                                                  CCGGAATTCGGCCCAGCCGGCCNNN-----NGA
                                                                                                                                                    CCGGAATFCGGCCCAGCCGGCCNNN-----NAA
                                                                                                 3. end for 40 VII genes
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    CGNNN-----G
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       GGNNN-----C
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              CCNNN------C
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  GGNNN -----G
                                                                             GGCCTTAAGCCGGGFCGGCCGGNNN-----N
                                                                                                                                                                            GGCCTTAAGCCGGGTCGGCCGGNNH-----N
                                                                                                                                                                                                                                                                           GGCCTTAAAGCCGGGTCGGCCGGNNN-----N
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        / DPL2+DPL3
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           +v318
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             DPL11
    clu
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                Gln
                                                                                                                                                                                                                                                                                                                                                                                               Pro
                                                                                                                                                                                                                                                                                                                                                                                                                     NNN-----CCT
                          ECORI / Sfil
                                                                                                                                                                                                                                                                                                                                                                           All kappa genes:
                                                                                                                                                                                                                                                                                                                                                                                                                                             GGNNN-----G
(except VK L20)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           Lambda 3 genes:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Lambda 1 genes:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                Lambda 3 gene:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Lambda 2 gene:
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10 20 30 40 50 60	
10 20 30 40 50 60 1234567890 1234567890 1234567890 1234567890	
CCCASCOG CCATGCCCCA GGIGCAGCIG GIGCAGICIG GGGAGGCIT GGIAAAGCCT	60
Q V Q L V E S G G G L V K P	~
O A O D A E 2 G G B A K I	
GREGGICCC TTAGACTCTC CIGIGCAGCC ICIGRATTCA CTITCAGIAA CGCCTGGATG	120
G G S L R L S C A A S G F T F S N A W M	
G G S L R L S C A A S G F I F S N A W H	
ACCIGGGICC GCCAGGCICC AGGGAAGGGG CIGGAGIGGG TIGGCCGIAT TAAAAAGCAAA	180
SWVRQAPGKGLEWVGRIKSK	200
SWVRQAPGRG LEWVGRIKSK	
ACTIGATOGTIG GGACAACAGA CTACGCTGCA CCCGTGAAAG GCACATTCAC CATCTCAAGA	240
T D G G T T D Y A A P V K G R F T I S R	2.0
T D G G T I D I A A P V R G R I I I D R	
CATGATTCAA AAAACACGCT GIATCIGCAA ATGAACAGCC TGAAAACCGA GGACACACCC	300
D D S K N T L Y L Q M N S L K T E D T A	300
GIGTATTACT GTACCACAGT TOCGITGICT GCCCACCGTG GCATGTGCCG TCAAGCAACT	360
V Y Y C T T V A L S A D R G M W G Q G T	200
V Y Y C T T V A L S A D R G H W G Q G I	
CTGGTCACCG TCTCCTCAGG TGGAGGCGGT TCAGGCGGAG GTGGCTCTGG CGGTGGCGGA	420
L V T V S S G G G G S G G G S G G G G G G G G	120
TOCCATCITIC TCATCACTCA CTCTCCACTC TCCCTGCCCG TCACCCTTGC ACACCCGCCC	480
S D V V M T Q S P L S L P V T L G Q P A	100
TOCATOTOCT GCAGGICTAG TOAAAGCCTO GTATACAGIG ATGGAAACAC CTACTIGAAT	540
SISCRSSQSLVYSDGNTYLN	
TOGITTCAGC AGAGCCAGG CCAATCTCCA AGGCGCCTAA TITATAAGGT TTCTAACCGG	600
WFQQRPGQSPRRLIYKVSNR	
CACTICTOGGG TCCCAGACAG ATTCAGCGGC AGTGGGTCAG GCACTGATTT CACACTGAAA	660
DSGVPDRFSGSGSGTDFTLK	
ATCACCAGGG TGGAGGCTGA GGATGITGGG GITTATTACT GCATGCAAGG TACACACTGG	720
I S R V E A E D V G V Y Y C M Q G T H W	
CCTTACACTT TIGECCAGGG GACCÁAGCTG GAGATCAAAG CGGCCGC	าธา
PYTF GQG TKL EIK	

10 20	60
1234567890 1234567890 1234567890 1234567890 1234567890 12345678	90
GCCCAGCCG CCATGCCCCA CATGCAGCTG GTGCAGTCTG GGGCAGGCTT GGTAAAGC	CT 60
Q M Q L V E S G G G L V K P	
GOGGGGTOCC TRAPACTOTO CIGIRCAGOO TOTOGRATICA CITICAGIAA COCCIGGA	IG 120
GGSL RLS CAA SGFT FSN AWM	
ACCIGOGICC GCCAGOCICC AGOGAAGOGG CIGGAGIGGG TIGGCCGIAT TAAAAAGCA	
SWVR QAP GKG LEWV GRI KSK	
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ACTIGATICGIG GGACAACAGA CTACGCIGCA CCCGIGAAAG GCAGATTCAC CATCTCAA	GA 240
TDGG TTD YAA PVKG RFT ISR	
CATCATTCAA AAAACACCCT GTATCTGCAA ATGAACAGCC TGAAAACCGA GGACACAG	CC 300
D D S K N T L Y L Q M N S L K T E D T A	
GIGTATTACT GTACCACATG GESCTOCGTC GACACGGACA ACTACGCCAG GITTTGGG	GT 360
V Y Y C T T W G S V D T D N Y A R F W G	
VIICIIW GSV DIDNIAR FW G	
CAAGGAACTC TGGTCACCGT CTCCTCAGGT GGAGGCGGTT CAGGCGGAGG TGGCTCTG	SC 420
QGTL VTV SSG GGG SGG GSG	
GGTGCCCGAT CCCACATCCA GATGACCCAG TCTCCATCCT CCCTGTCTGC ATCTGTAG	SA 480
G G G S D I Q M T Q S P S S L S A S V G	
GACAGAGTCA CCATCACTTG CCGGGGGAGT CAGGGCATTA GCAATTATTT AGCCTGGT	AT 540
DRVT ITC RAS QGIS NYL AWY	
D K V I I I C K A S Q G I S N I L A W I	
CASCAGAAAC CASGGAAAGT TOCTAAGCTC CTGATCTATG CTGCATOCAC TTTGCAAT	CA 600
QQKPGKVPKLLIYAASTLQS	
GCCCTCCCAT CTCCCTTCAG TGCCAGTCCA TCTCCCCACCA ATTTCACTCT CACCATCA	SC 660
G V P S R F S G S G S G T D F T L T I S	
ACCCTGCAGC CTGAAGATGT TGCAACTTAT TACTGTCAAA AGTATAACAG TGCCCCTG	rc 720
S L Q P E D V A T Y Y C Q K Y N S A P L	
ACTITICECCE CASCEACCAA CETOGAGATC AAACCCCCCCE C	761
T F G G G T K V E I K	

10 20 30 40 50	60
1234567890 1234567890 1234567890 1234567890 1234567890 12	34567890
GCCCAGCCGG CCATGGCCCA CATGCAGCTG GTGCAGTCTG GGGCAGGCTT GG	TAAAGCCT 60
Q M Q L V E S G G G L V	K P
GEGEGGICCC TTAGACICIC CIGIGCAGCC TCIGGATICA CITTCAGIAA CO	CCTGGATG 120
GGSLRLSCAASGFTFSNA	W M
ACCIGGGICC OCCAGGCICC ACCGAAGGGG CIGGAGIGGG TIGGCCGTAT TA	AAAGCAAA 180
SWVRQAPGKG LEWV GRIK	S K
ACTGATGGTG GGACAACAGA CTAGGCTGCA COCGTGAAAG GCAGATTCAC CA	TCTCAAGA 240
T D G G T T D Y A A P V K G R F T I	SR
	200
GATGATICAA AAAACAGGCI GIATCIGCAA AIGAACAGCC IGAAAACCGA GG	
D D S K N T L Y L Q M N S L K T E D	TA
	AAGGAACT 360
GIGTATIACT GTACCACACC GGGCTGGTAT GGGGCCGAGG ATAAGTGGGG TC	
V Y Y C T T P G W Y G A E D K W G Q	G T
	GTGGCGGA 420
CICCICACCG TCICCICACG TCCACCCCGT TCACCCCCAG GICCCTCTGG CC	
	GG
TOCGACATOC AGATGACOCA GICTOCATOC TOCCIGICIG CATCIGIAGG AG	ACAGAGTC 480
S D I Q M T Q S P S S L S A S V G D	
	TC V
ACCATCACTT GCCGGGCGAG TCAGGGCATT AGCAATTATT TAGCCTGGTA TC	AGCAGAAA 540
TITCRASOGISNYLAWY Q	
	2
CCAGGGAAAG TTCCTAAGCT CCTGATCTAT GCTGCATCCA CTTTGCAATC AG	GGGTCCCA 600
PGKVPKLLIYAASTLQSG	
TCTCGGTTCA GTGGCAGTGG ATCTGGCACA GATTTCACTC TCACCATCAG CA	GCCTGCAG 660
SRFS GSG SGT DFTL TIS S	
	_
CCTGAAGATG TTGCAACTTA TTACTGTCAA AAGTATAACA GTGCCCCTTT CA	CTTTCGGC 720
PEDV ATY YCQ KYNS APF T	' F G
CCTCCCACCA AAGTCCATAT CAAAGCCCCC CC	752
PGTK VDI K	

10 20 30 40 50 60	
1234567890 1234567890 1234567890 1234567890 1234567890	60
GCCAGCGG CCATGCCCA GGIGCAGCIG GIGCAGICIG GGGCAGCTT GGIACAGCT	ω
Q V Q L V E S G G G L V Q P	
CLEARING TEACACTOR CIGICOSCO TOTOGATICA COTOGATAA CAGTGACATO	120
G G S L R L S C A A S G F T F S N S D M	220
G G S L R L S C A A S G F I F S N S D N	
AACTIGGGTCC ATCAGGCTCC AGGAAAGGGG CTGGAGTGGG TATCGGGTGT TAGTTGGAAT	180
N W V H Q A P G K G L E W V S G V S W N	
NWVHQAPGRG LLWV 50 V 5 W I	
GCCAGTAGGA CCCACTATGC AGACTCTGTG AAGGCCCGAT TCATCATCTC CAGAGACAAI	240
G S R T H Y A D S V K G R F I I S R D N	
G S R I I I I B S I II C II S	
TCCAGGAACA COCTGTATCT GCAAACGAAT AGOCTGAGGG COGAGGACAC GOCTGTGTAT	300
SRNT LYL QTN SLRA EDT AVY	
TACTOTOTO CAACCOATOS COAGTOGTAC COCOCCTOGO GTCAACCAAC TCTCGTCACC	360
Y C V R T D G E W Y G A W G Q G T L V T	
GICTOCTCAG GIGGAGGGG TICAGGGGA GGIGGCTCIG GCGGIGGGG ATCCGCCATC	420
V S S G G G S G G G G G G S A I	
CAGATGACCC AGTICTCCATC CTCCCTGTCT GCATCTGTAG GAGACAGAGT CACCATCACT	480
QMTQSPSSLSASVG DRV TIT	
	F40
TOCCOGCCHA GTCAGGGCAT TAGAAATGAT TTAGGCTGGT ATCAGCAGAA ACCAGGGAAA	A 540
CRAS QGIRND LGWY QQK PGK	
and the second s	600
GCCCCTAAGC TCCGGATCTA TGCTGCATCC AGTTTACAAA GTGGGGTCCC ATCAAGGTTC	. 600
APKL RIY AAS SLQS GVP SRF	
ACCESCACIG GATCTESCAC AGATTICACT CTCACCATCA GCAGCCIGCA GCCTGAAGAI	660
S G S G S G T D F T L T I S S L Q P E D	. 000
TTTGCAACTT ATTACTGTCT ACAAGATTAC AATTACCCTC TCACTTTCGG CGGAGGGACC	720
FATY YCL QDY NYPL TFG GGT	
AAGGTGC AGA TCAAAGCCCC CCC 1	743
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				120
ا کا تا تا	Y K L S	CAG	S G F T F S S Y A M	
			CIGGAGIGGG TATCABCIAT IGGIACIGGT	180
HWVI	QAP	G K G	LEWV SAI GTG	
GGTGGCACAC	ACTATICAÇÃ	CTCCGTGAAG	GECCGATTCA CCATCTCCAG AGACAATCCC	240
			G R F T I S R D N A	240
G G 1 .		S V K	G R F I I S R D N A	
			CTGAGAGCCG AGGACATGCC TGTGTATTAC	300
KNSI	YLQ	M N S	L R A E D M A V Y Y	
TGTGCAAGAT	GGGGGCACGT	CGCTTGTGG	GTTGCGGACG TCTATTGGGG TCAAGGAACT	360
CARV			V A D V Y W G Q G T	200
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دستحسم محد		man anama	mai access a company	
			TCAGGGGAG GIGGCICIGG COGIGGCGGA	420
LVT	SSG	GGG	S G G G G G G G	
			1	
TCCGACATCC	AGATGACCCA	GICTOCATOC	TOCCIGICIG CATCIGIAGG AGACAGAGIC	480
			S L S A S V G D R V	
	~			
ACCEPTE CTT	CHARTER	الشعاعية والمعاشية	ASCAATTATT TAGOCTGGTA TCAGCAGAAA	540
T T T C	D A C	0.0.7		540
1 1 1	r a s	QGI	SNYLAWYQQK	
CCAGGGAAAC	TICCIAAGCI	CCIGAICIAT	GCTGCATCCA CTTTGCAATC AGGGGTCCCA	600
PGKV	PKL	T T 🗸	A A S T L Q S G V P	
			A A S I L Q S G V P	
TCTCCGTTC				660
TCTCGGTTCA	GTGGCAGTGG	ATCTGGGACA	CATTICACIC TCACCATCAG CAGCCTGCAG	660
TCTCGGTTCA S R F S	GTGGCAGTGG	ATCTGGGACA		660
SRFS	GTGGCAGTGG G S G	ATCTCCCACA S G T	CATTICACIC TCACCATCAG CACCCTCCAG D F T L T I S S L Q	
S R F S	CTCCCACTTCC G S G	AICIGGGACA S G T TIACIGICAA	CATTICACIC TCACCATCAG CACCCIGCAG D F T L T I S S L Q AAGIATAACA GIGCCCCTTA CACTITIGGC	660 720
S R F S	CTCCCACTTCC G S G	AICIGGGACA S G T TIACIGICAA	CATTICACIC TCACCATCAG CACCCIGCAG D F T L T I S S L Q AAGIATAACA GIGCCCCTTA CACTITIGGC	
S R F S CCTGAAGATO P E D V	GTGGCAGTGG G S G TTGCAACTTA A T Y	ATCTGGGACA S G T TTACTGTCAA Y C Q	GATTICACIC TCACCATCAG CAGCCIGCAG D F T L T I S S L Q AAGIATAACA GIGCCCCTTA CACTTITIGGC K Y N S A P Y T F G	
S R F S CCTGAAGATO P E D V	GTGGCAGTGG G S G TTGCAACTTA A T Y	ATCTGGGACA S G T TTACTGTCAA Y C Q	GATTICACIC TCACCATCAG CAGCCIGCAG D F T L T I S S L Q AAGIATAACA GIGCCCCTTA CACTTITIGGC K Y N S A P Y T F G	720
S R F S CCTGAAGATG P E D V CAGGGGACCA	CTCCCACTTCC G S G	ATCIGGGACA S G T TIACIGICAA Y C Q CAAAGCGGCC	GATTICACIC TCACCATCAG CAGCCIGCAG D F T L T I S S L Q AAGIATAACA GIGCCCCTTA CACTTITIGGC K Y N S A P Y T F G	

10	20	30	40	50 60	
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COLYGOUS:	CATTGGCA	GATICCACCTG	GIGCAGICIG	GCCGAGCCTT GGTACAGCCT	60
GCC 2CCC	0	M O T	V O S G	G G L V Q P	
	¥	2 2	. 2		
			marchines.	CCTTCAGTAG CTATCCTATG	120
Garagicco	16454C1C1C	. CIGIGLAGE	C C E M	E C C V N M	120
GGSL	R L S	CAG	5 G F 1	F S S Y A M	
					100
CACTGGGTTC	GCCAGGCTCC	: AGGAAAAGGI	CICCAGICCC	TATCAGCTAT TOGTACTOGT	180
H W V R	Q A P	GKG	LEWV	S A I G T G	
GGTGGCACAT	ACTATGCAGA	. CTCCGTGAAG	GGCCGATTCA	CCATCTCCAG AGACAATCCC	240
G G Т Y	Y A D	s v K	GRFT	ISRDNA	
2202200000	מינודי אוי בינדי אוי	בשבשבשבב	المستحصصين	AGGACATOSC TGTGTATTAC	300
				D M A V Y Y	300
KNZL	r L Q	ri iv 5	пиип	DHAVII	
				cmos soos so momormos co	200
				GICAAGGAAC TCIGGICACC	360
CARE	G E L	GVT	SFWG	QGTLVT	
				GCGGTGGCGG ATCCGACATC	420
V S S G	G G G	S G G	G G S G	GGGSDI	
CAGATGACCC	AGTOTOCATO	CTCCCTGTCT	GCATCIGTAG	GAGACAGAGT CACCATCACT	480
				DRVTIT	
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ترکینی است	CICECCET	י הבתרבבידהי	ىلتكىلىكى تىلىك	ATCAGCAGAA ACCAGGGAAA	540
				O O K P G K	210
CAAS	Q G 1	2 1/1 ;	LAWI	QQRFGR	
CTTTCCTT3 3 CC	man series	m~m~m~	2000000000000	CZCZZZZZZZZZZZZZZZZZZZZZZZZZZZZZZZZZZZ	600
				CAGGGGTCCC ATCTCGGTTC	600
VPKL	T T X	A A S	TLQS	GVPSRF	
				GCAGCCTGCA GCCTGAAGAT	660
SGSG	SGT	DFT	LTIS	SLQPED	
GTTGCAACTT	ATTACTGTCA	AAAGTATAAC	AGIGCCCCIT	GCACGITCGG CCAAGGGACC	720
VATY	Y C Q	K Y N	SAPW	TFGQGT	
	~				
AAGGIGGAAA	TCAAAGCGGC	ac -			743
KVEI					, 1
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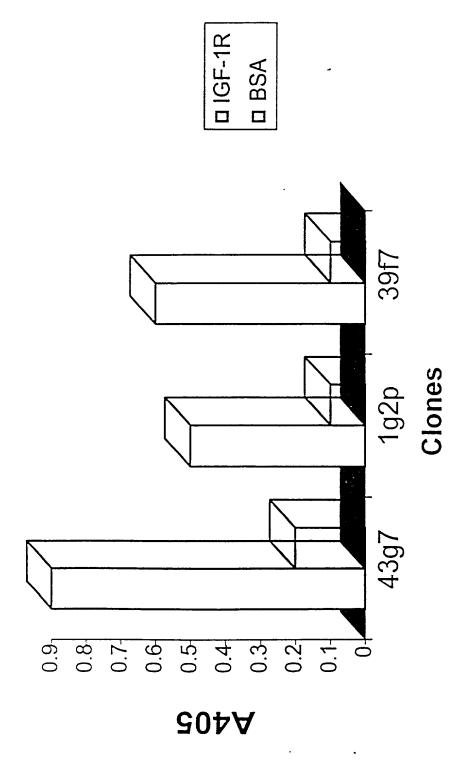


FIGURE 40

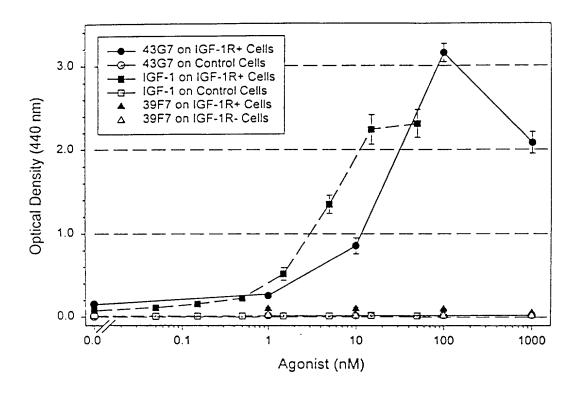
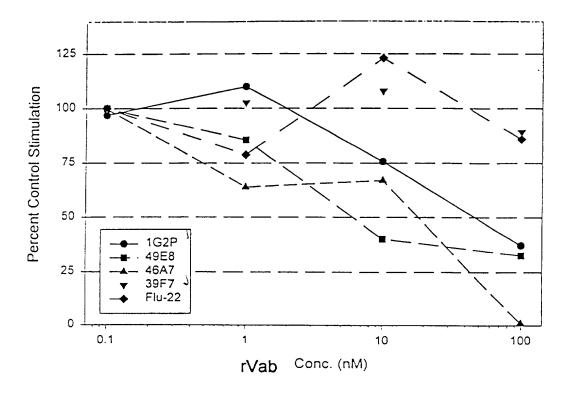


FIGURE 41



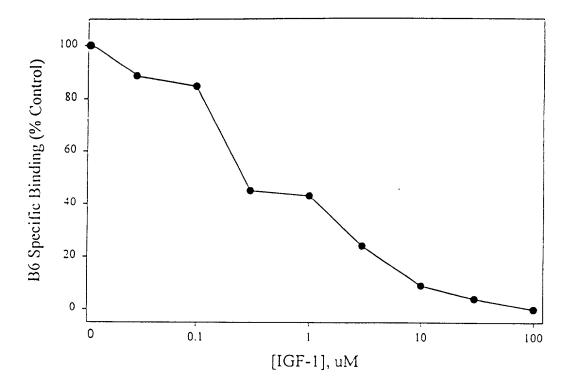


FIGURE 43

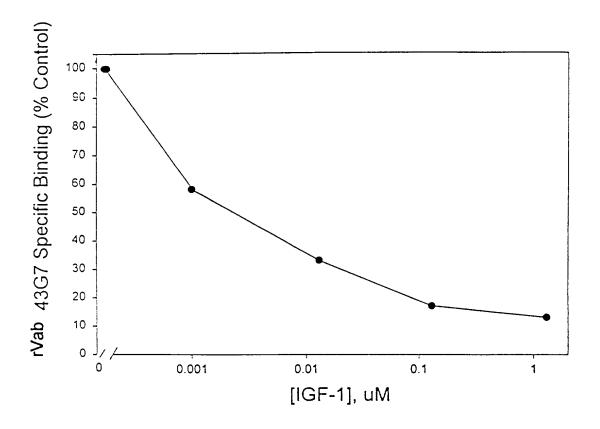


FIGURE 44

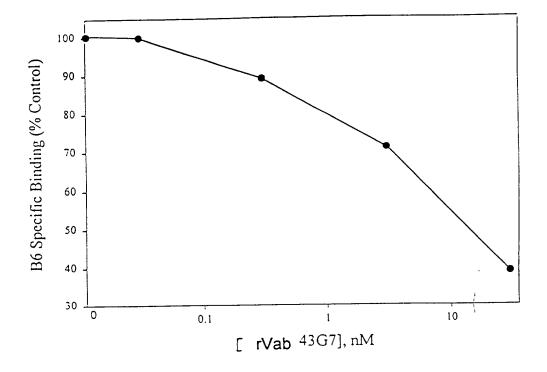
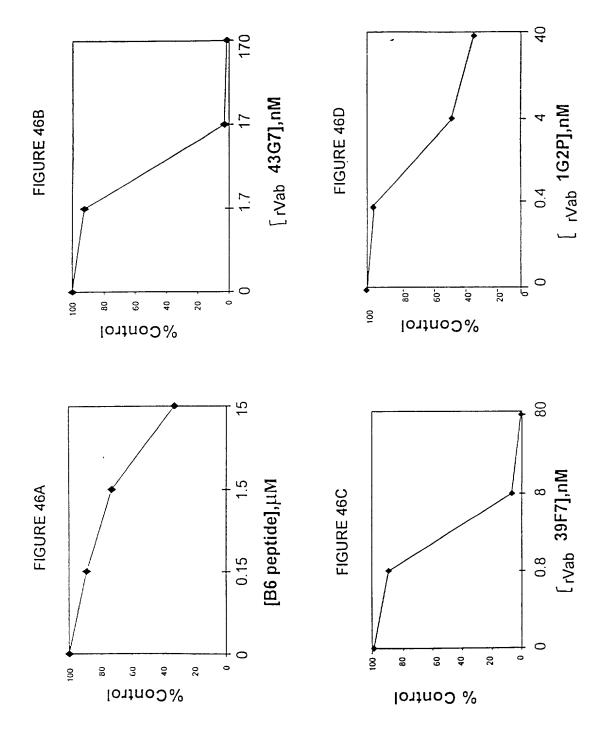


FIGURE 45



			Target	šct
		Found	R	IGF
: Formula 1 Motit	IGGOGOHODGNEYDWEVEALA	18	+	++++
	VFWNCRSOOLDFYEWFEQAA	16	+	++++
	RGGTEYEWEESALRKHGAG	œ	+	+++
	RVAGATSAPGLVSNKODGLEYSWERE	ഹ	+	+++
	VIOLENSINGED	4	+	++++
	DPERMOSDVGFYEWFRAAVG	က	+	+++
	WSALLSVMDTGEYAWEDDAV	2	++	++++
	MERMARIAN	2	+	+++
	IGGSFVEFYGWFNDQV	7	+	+++
	CHSWALVBRUEYEWEDL	7	++	+ + +
	LPAGGAOGFAVRGFYEWFES	7	+	+++
	RDKPTDOEEONWSFYEWFRII	-	+	+++
	SROOTNETENSAGEYGWEER	-	+	+++
	GAFYRWEIIEALVGSERVPDV	-	÷	+ + +
	RIGGGWARSEGFYEWFVREL	7	+	+
	RMFYEWFWSOMGAGPTEGSA	-	+	+
	HEAFYDWESALVDGGYELMG	1	+	++
	FYGWESROLSLTPRDDWGLP	. 1	+	+
	CVGTI.TMSSDAFYTWEV	- -1	+	++
	SOMMONERARIANTO	1	÷	÷
	1	က	+	++++
DIANANIG	1 2	~	+	++
40B2 TRDMHYVWVQI	TRDMHYWWQINDA TINGWAZA TASKATA SAN TASKATA	٢	+	+

WIDDEWAWVOCEVYGRGCPS EIEAEWGPVRCLVYGRCVGG Group 2: Formula 6 Motif 20A4*

DYKDFYDAIDQLVRGSARAGGTRD DYKURLFYCGIQALGANLGYSGCV DYKDDRAFYNGLRDLVGAVYGAWD DYKDFYSALWGLCGVTGCG RGOSDAFYSGLWALIGLSDG Group 3: Formula 2 Motif 20E2 20C11 20A12

Group 5: Miscellaneous Motif 10

WWWGGRNRWWI,FRWGLGGER PFGFGGRWWGIPRMWYRNS

Group 4 and 6: Miscellaneous Motif 10

WPGYLFFEEALQDWRGSTED CRVALMGPVWPRWWFMSRPV SMFVAGSDRWPGYGVLADWL VRGFOGGTVWPGYEWLRNAA LGPLLRWGSEVCGVWPDLCE

Group 7: Formula 4 Motif

HI.CVI.EFLEWGASLFGYCSG ACSSFFVKGPEGFLQCLGSI 40D6 FERGRGI,RTAMOI,MRRPRDWHFPHSLFWGAPPPLSG

Group 8: Non-Aligning Miscellaneous Sequences

Target	IGF	0	٠.
Ta	IR	+++	+++
	Found	13	۳

1,9	IR IGF	++++	++++	++++	++	+++
3	R	+	+	+	+	+
	Found	٦		-	П	7

	IGF	+	+
•	IR	+++	+
	Found	1	-

get	IGF	0	+	٠.	+	1-+
Target	IR	++	++	+ +	++	0
	Found	Э	-1	7	-	-

.äct	IGF	+	+	c
Target	K	+++	0	c
	Found	4	Н	-

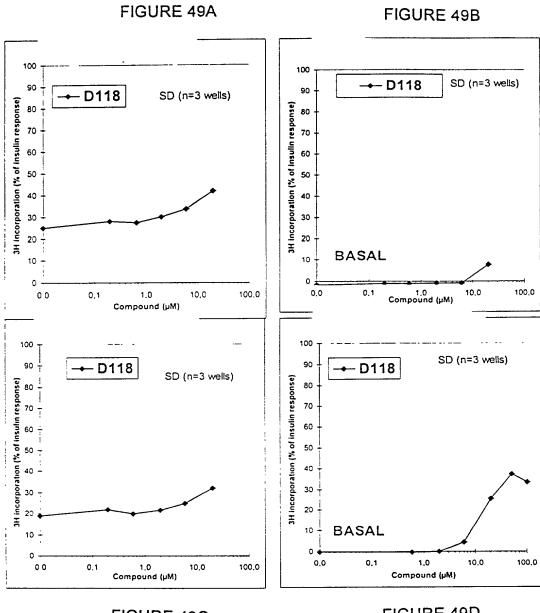
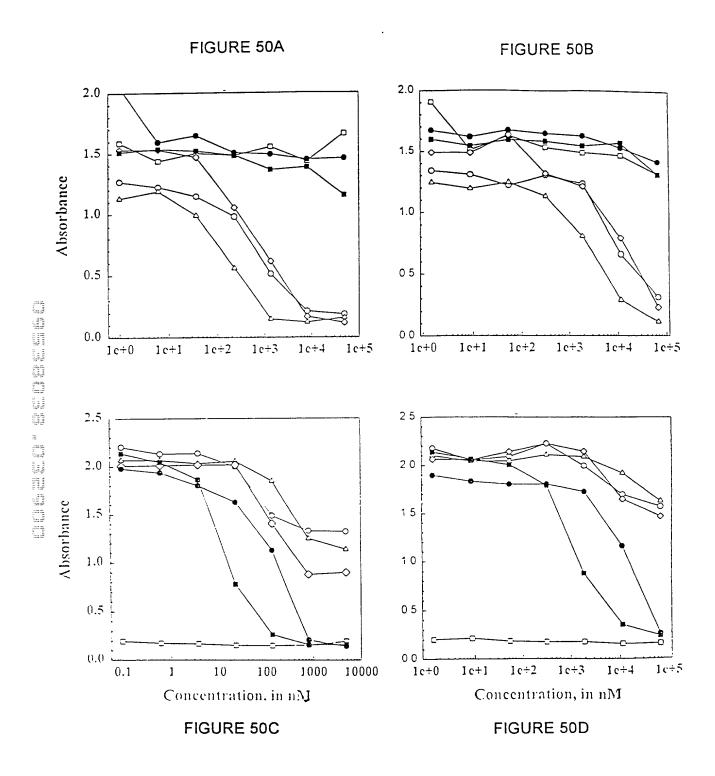
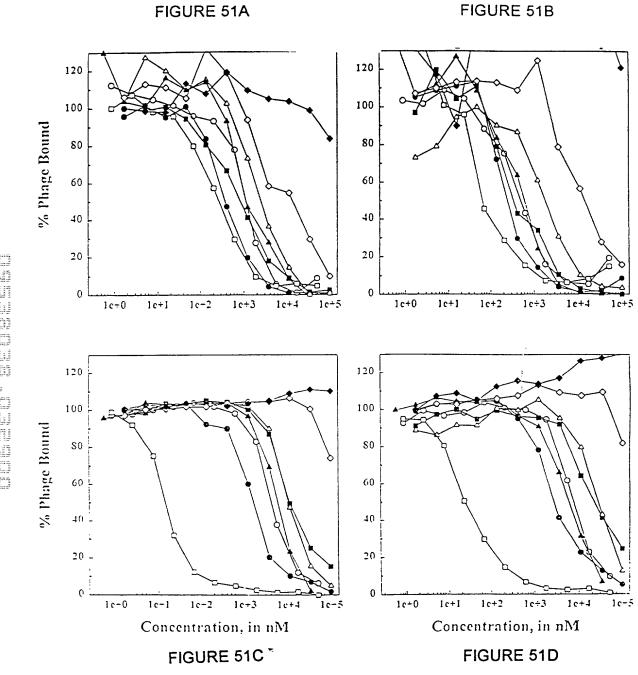
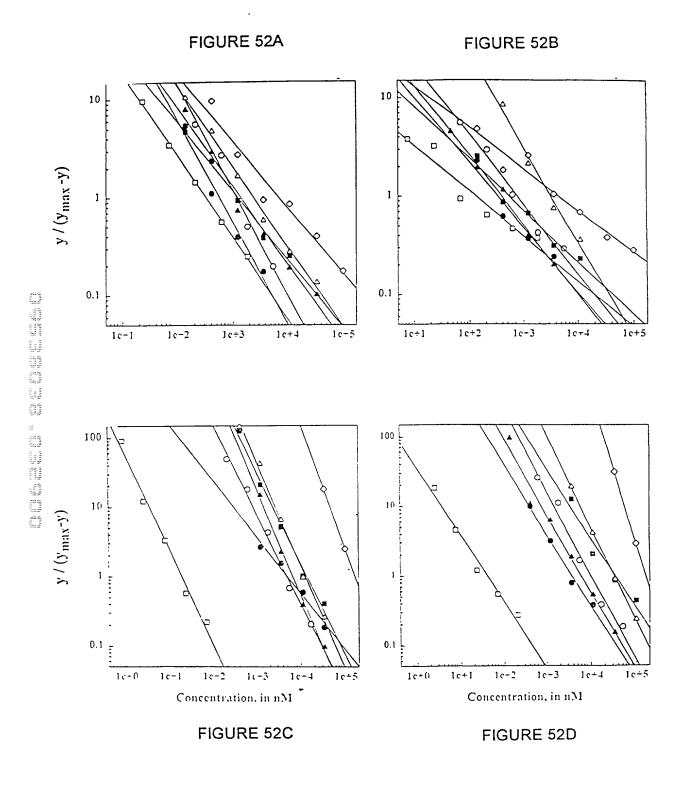


FIGURE 49C

FIGURE 49D







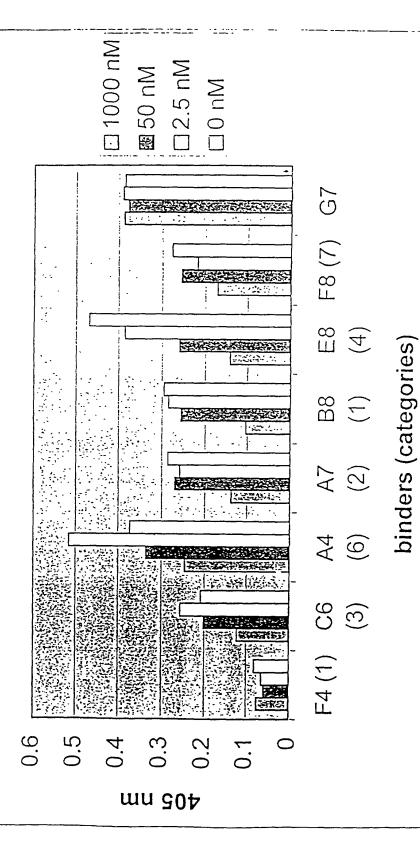


FIGURE 53

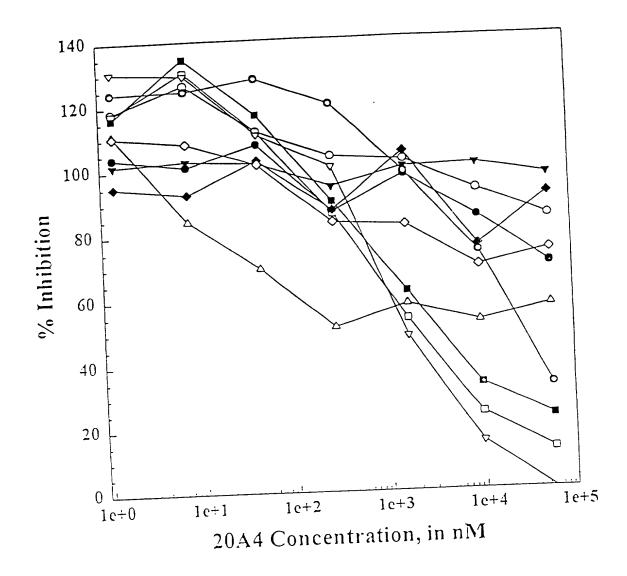


FIGURE 54

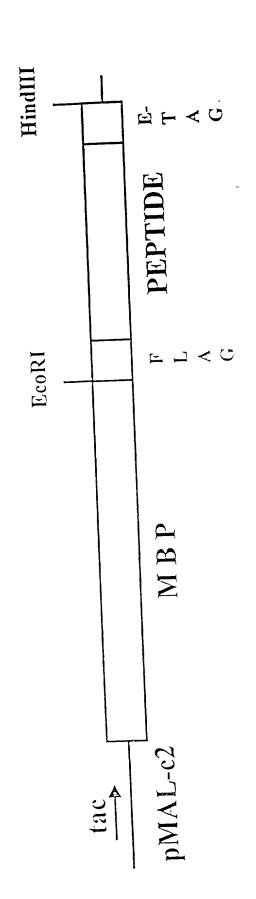
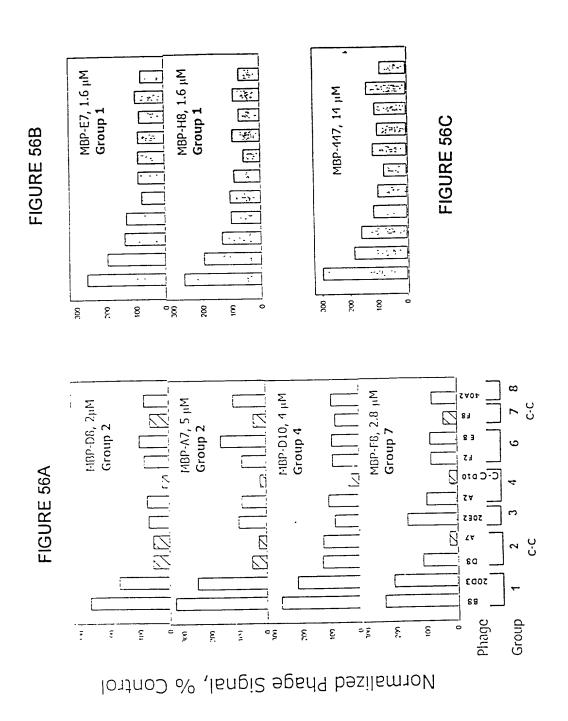


FIGURE 55

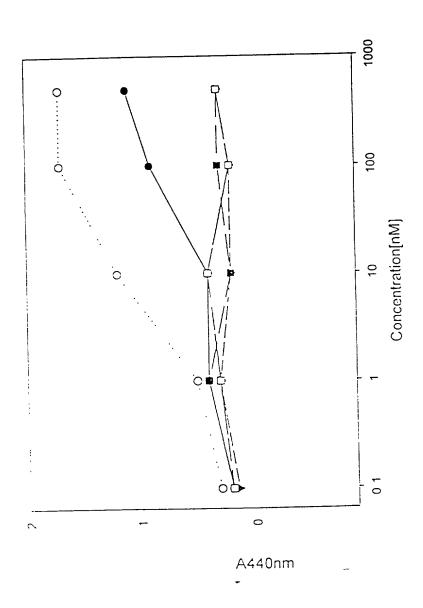


10 1234567890 12345678	20 30				
CCCCAGCCGG CCATGGCC					60
	E V Q L	VESG	GGL	VKP	
GGGGGTCCC TTAGACTC	TTC CTGTGCAGCC	TCTGGATTCA	CITICAGIAA	CCCCTCCATC	120
G G S L R L					
			•		
AGCTGGGTCC GCCAGGCT	TOC AGGGAAGGGG	CTGGAGTGGG	TIGGCCGTAT	TAAAAGCAAA	180
S W V R Q A					
ACTIGATIGGTG GGACAACA	AGA CTACGCTGCA	CCCGTGAAAG	GCAGATTCAC	CATCTCAAGA	240
TDGGTT	D Y A A	PVKG	R F T	ISR	
GATGATTCAA AAAACAC	GCI GTATCIGCAA	ATGAACAGCC	TGAAAACCGA	GGACACAGCC	300
D D S K N T	L Y L Q	MNSL	K T E	DTA	
GIGIATIACI GIACCACI	ATA OGGOGACGIT	TACGACCGCG	ATTACGATGG	CCCCTCCCCT	360
V Y Y C T T					
CAAGGAACTC TGGTCACC	TERASTEOTE TEC	CGLCCCCTT	CHESCOSCHES	TESETETESE	420
Q G T L V T	V S S G	G G G S	G G G	G S G	
				,	
GEIGEGERT COGRACATO	CA GATGACCCAG	TOTOCATOOT	COCTGICTGC	ATCTCTAGGA	480
G G G S D I					
CHCHCHGTCH CCHTCHGT	TELECESSON STI	CAGGGCATTA	GCAATTATTT	AGCCTCGTAT	540
D R V T I T	C R A S	Q G I S	\mathbb{H} X Γ	$\mathbb{A} \setminus U \setminus Y$	
CHOCHOHARC CAGGORA					600
QQXPGX	V P K L	P I A Y	A S T	L Q S	
GBBGTCCCAT CTCGGFTC					660
G V P S R F	S G S G	SGTD	FTL	TIS	
AGOTTGCAGO CTGARGA					720
SLQPED	V A T Y	Y C Q K	λ 11 Z	A P F	
YOUTHOGSOO GIGGSFOO			С		761
T F G P G T	H V D I.	K			

			10			20			30				40			50			60	
12	345	678	90	1234	567	7890	123	456	7890	12	345	678	90	1234	567	890	123	456	7890	
GC	CCA.	Œ	Œ	CCAT	GGC	.CC-2.	GGI	GCA	GCTG	TI	GGA	GTC	TG.	GGGG	AGC	CTT	GGI	ALL	CCT	60
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AC	TGA.	.TGG	TG	CGAC	AAC	ACT.	CTA	.CGC	TGCA	$\alpha$	CGI	Cyy	ÆG	CCAC	ATT	CAC	CAT	CIC	ARGA	240
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S	$\supset$	~	Q	L	Ţ	Q	S	P	S	F	ī.	S	<i>.</i> ~.	S	V	G	D	F.	7.	
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CC	733	34.0	<u>_=</u> ,	AAGT	33%	TAT	C	<b>-</b> GX	3333	GC?	33.	TGC	_							758
				7,-				•	•											

### CLONES VHCDR3 %Inhibition Activity

?	118:	PFFV	FYRGODI	548
	InsulB: 12H10:	FVNQH <u>LCG</u> SH <u>LVEALY</u> L <u>V</u> C VVYNYA	<del></del>	428
Ang?	13-e-4:	VQAMDG $L$	G RES	52%
?	13h9:	GGL	G RRDWL	30%
•	24:	GGRR	H RLG	
?	InsulinA lla8:	GIVEQCCTSICSLYQ LENY ENY	<u>C</u> n Gnse	32%
U/A		GDØSTÖNA		None



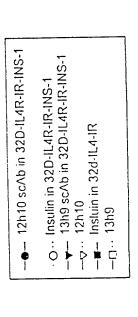


FIGURE 60

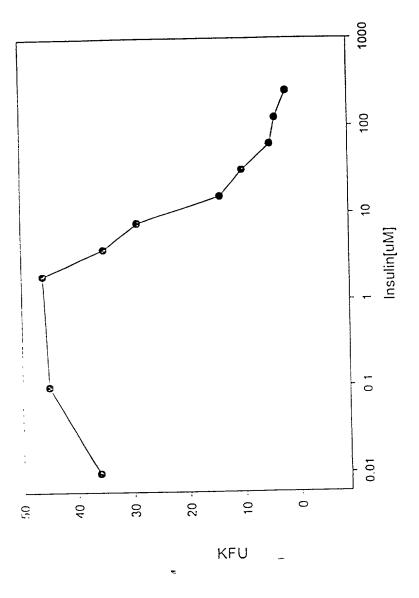


FIGURE 61

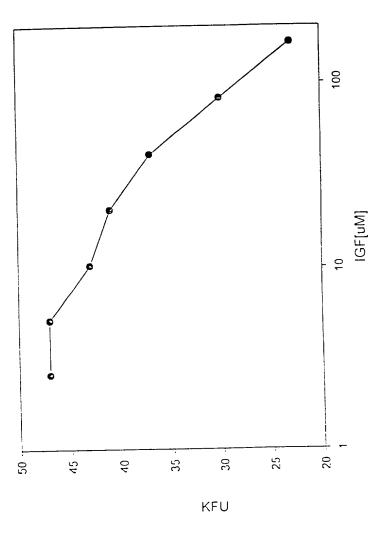


FIGURE 62



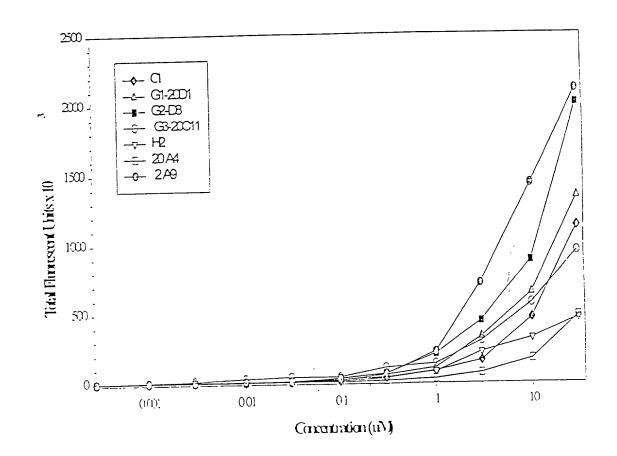


FIGURE 63

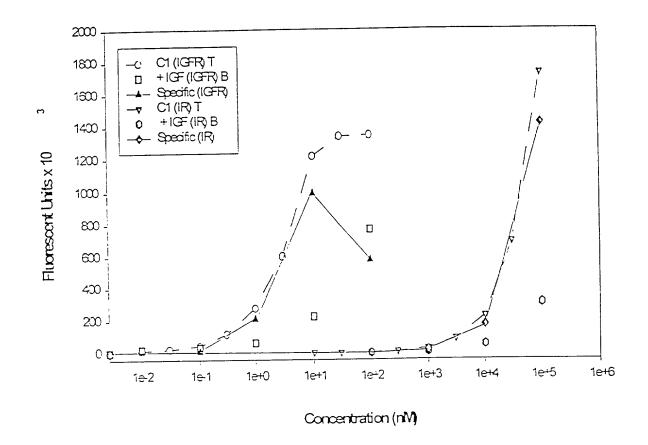


FIGURE 64

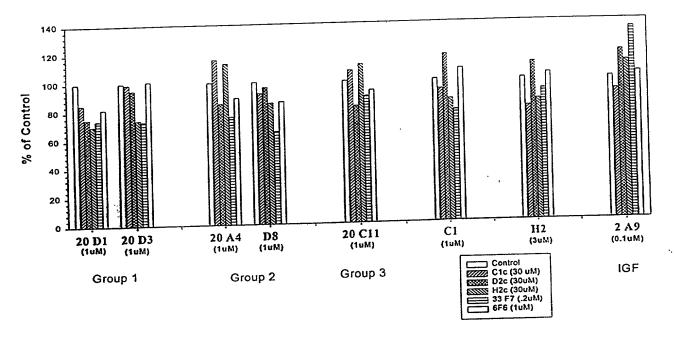


FIGURE 65

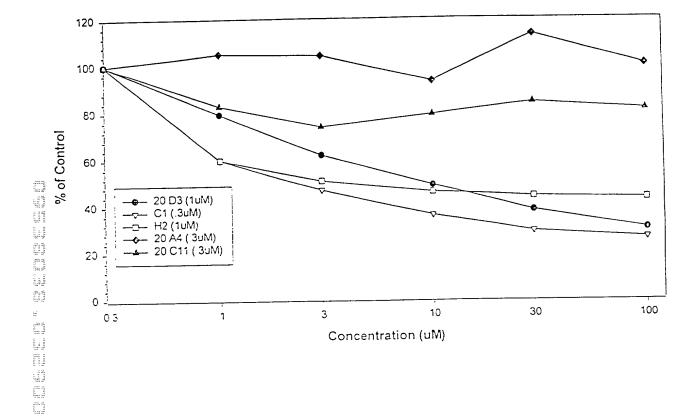


FIGURE 66

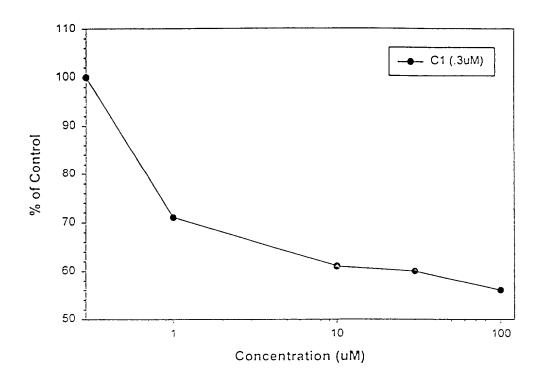


FIGURE 67

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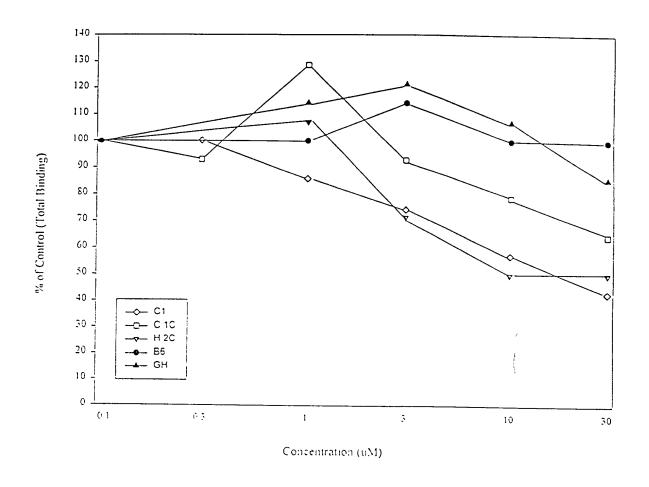


FIGURE 68

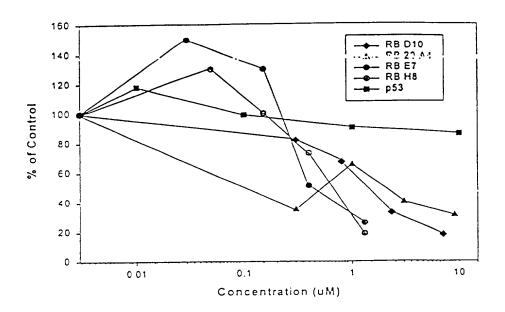
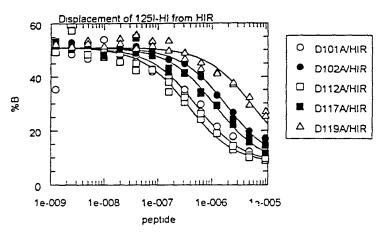
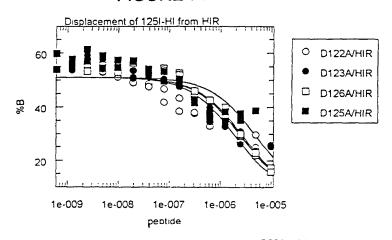


FIGURE 69

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## FIGURE 70A D990114A



## FIGURE 70B D990118A

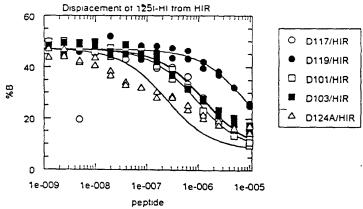
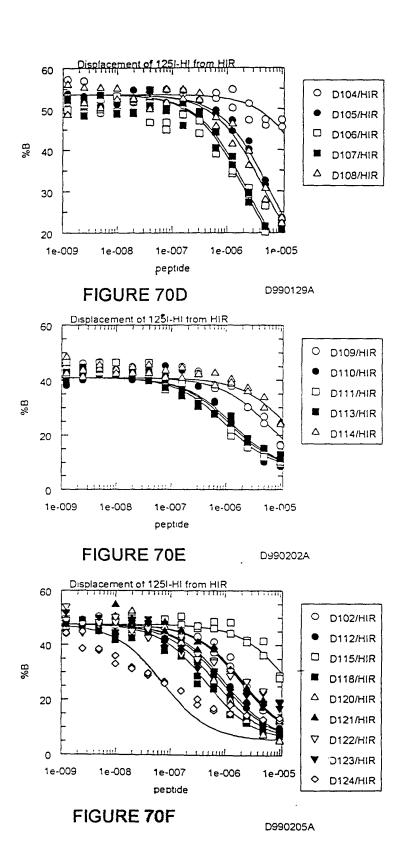
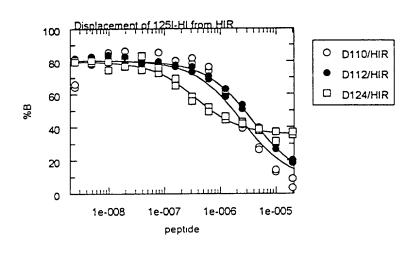
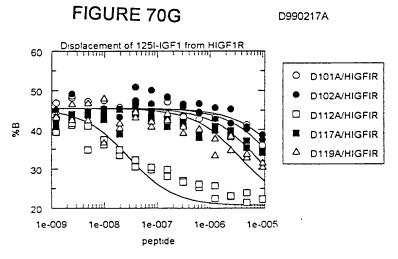


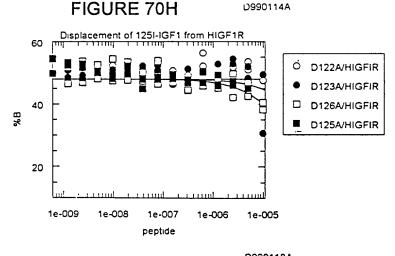
FIGURE 70C

D990126A



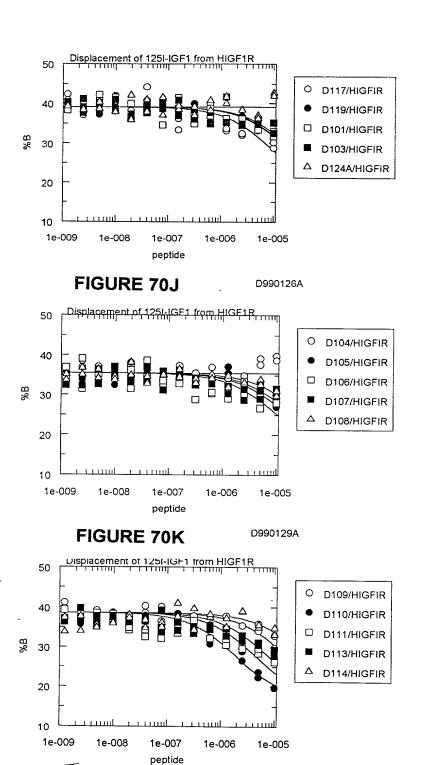






D990114A

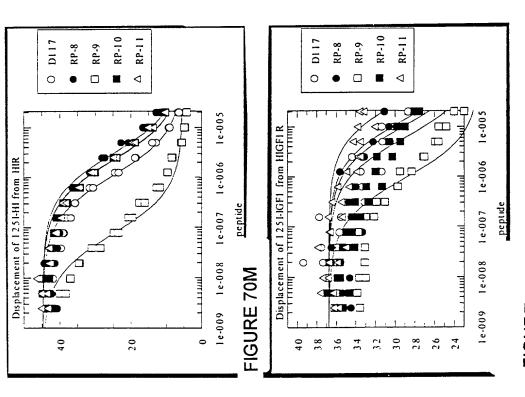
D990118A FIGURE 701



D990202A

FIGURE 70L

)



**FIGURE 70N** 

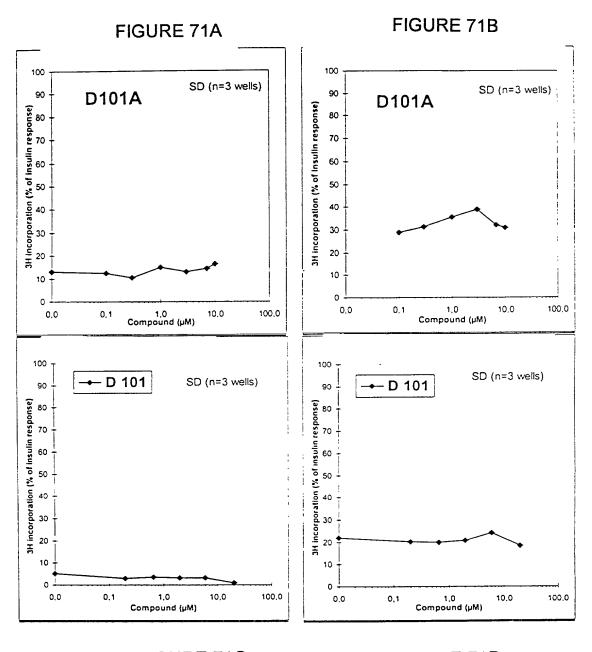
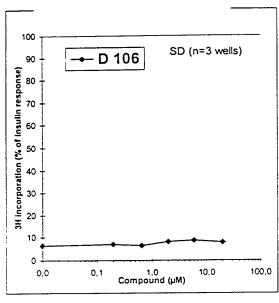


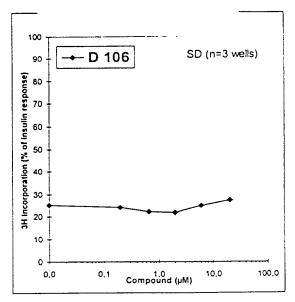
FIGURE 71C

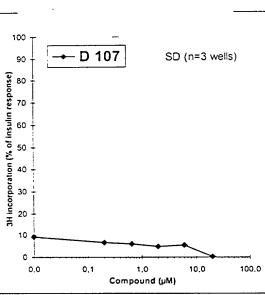
FIGURE 71D



### FIGURE 71F







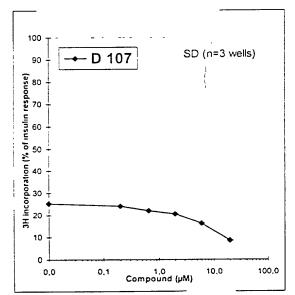


FIGURE 71G

FIGURE 71H

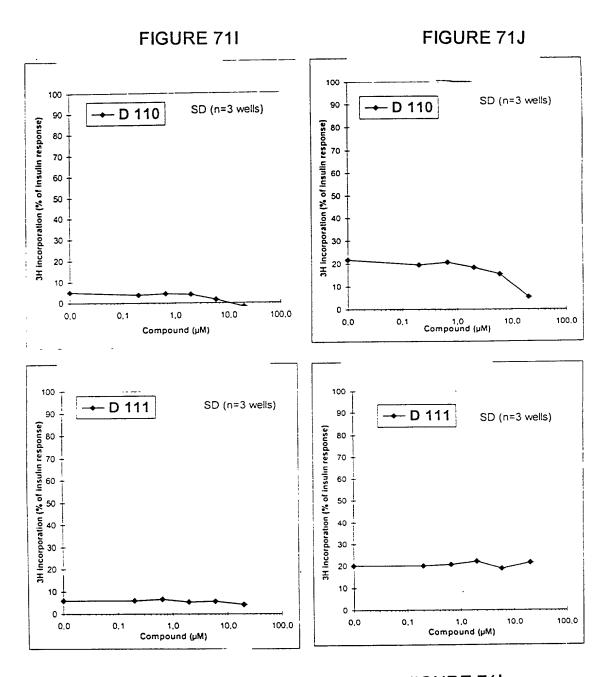


FIGURE 71K

FIGURE 71L

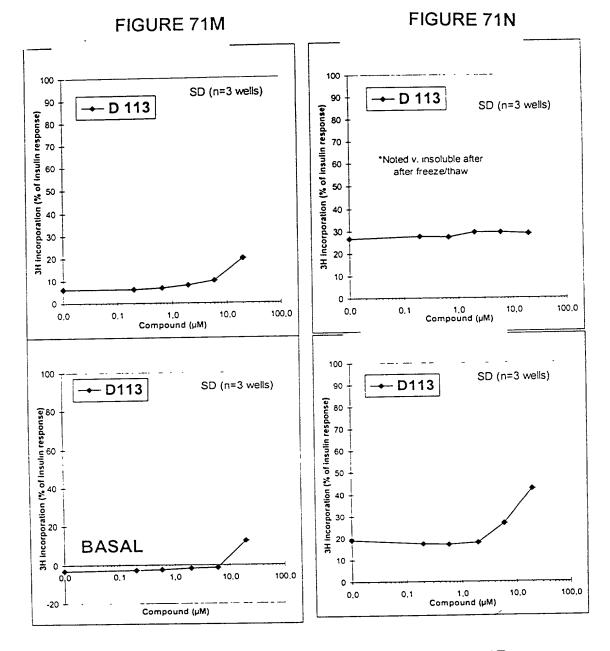


FIGURE 710

FIGURE 71P

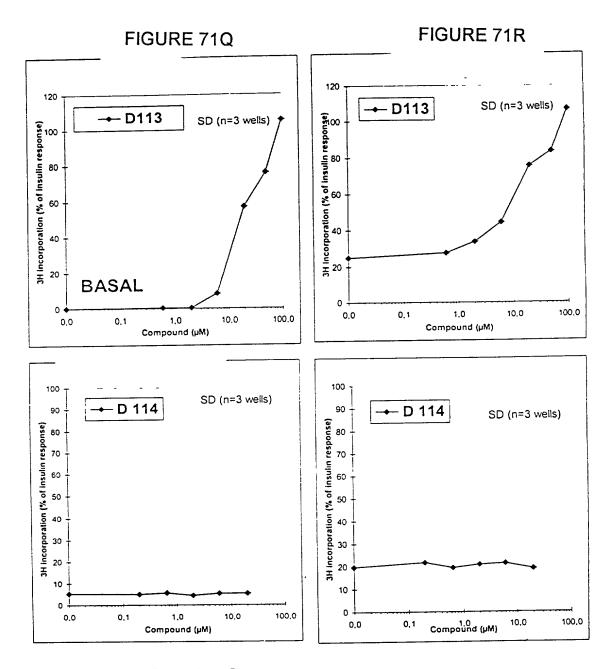


FIGURE 71S

FIGURE 71T

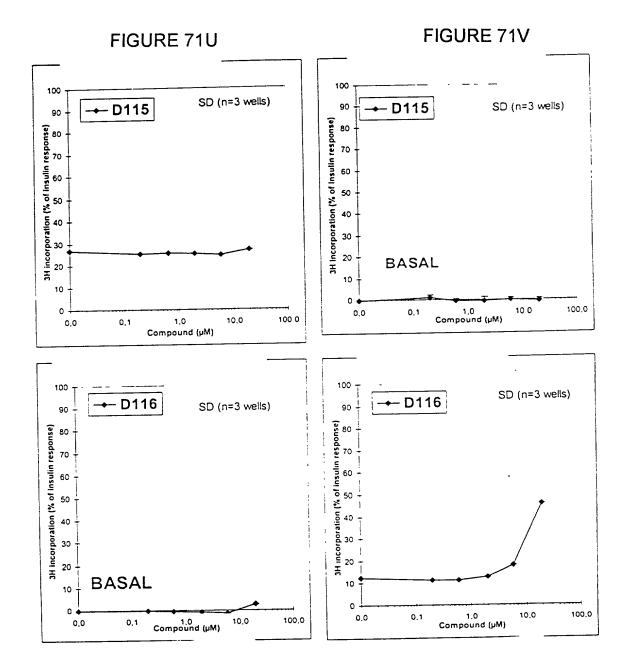


FIGURE 71W

FIGURE 71X

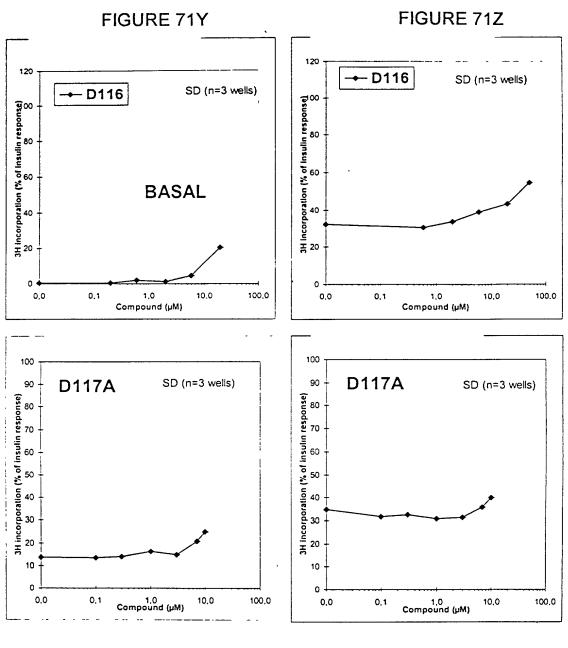


FIGURE 71A2

FIGURE 71B2

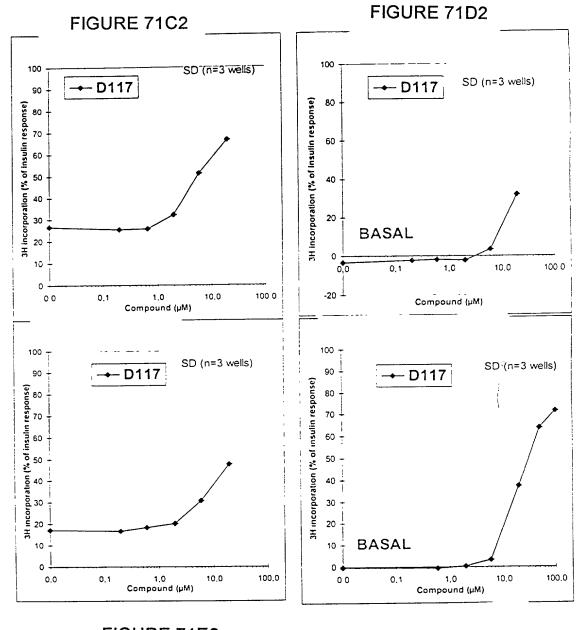
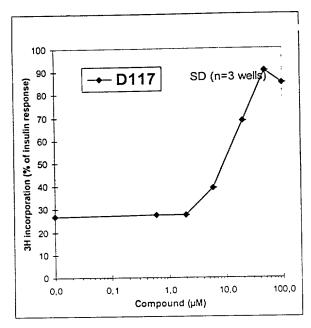
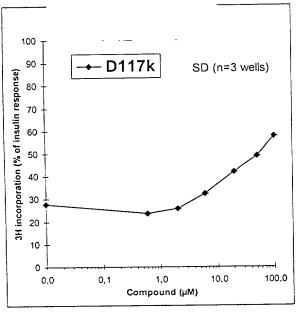


FIGURE 71E2

FIGURE 71F2

FIGURE 71G2





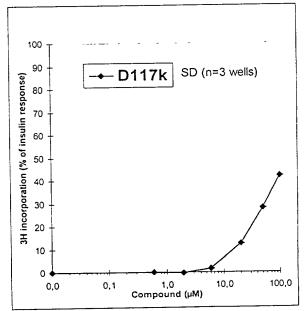
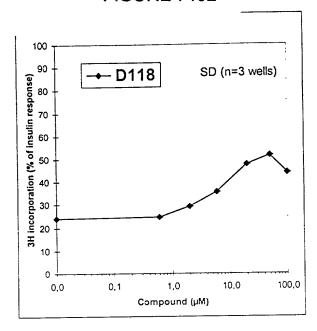
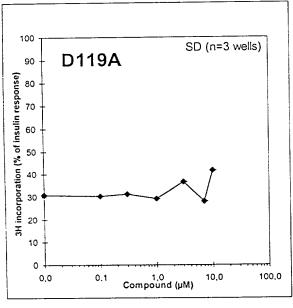


FIGURE 71H2

FIGURE 7112

FIGURE 71J2





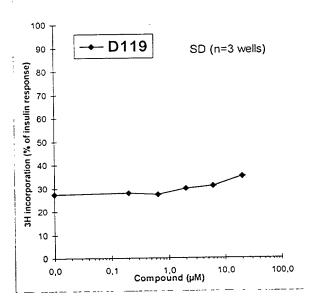


FIGURE 71K2

FIGURE 71L2

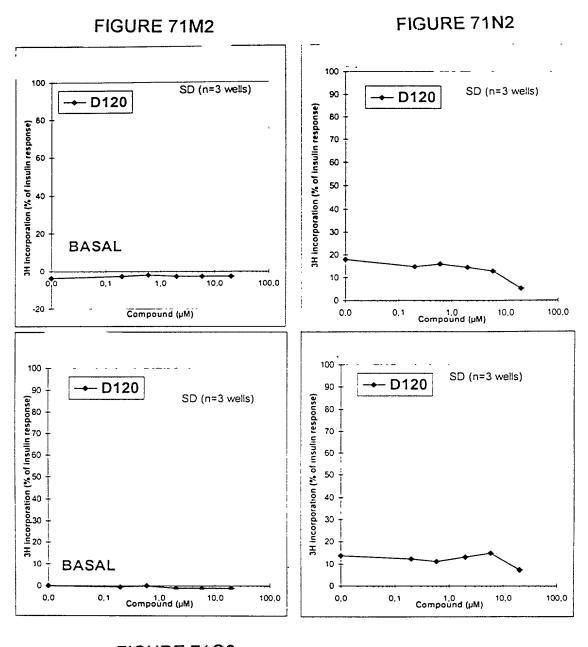
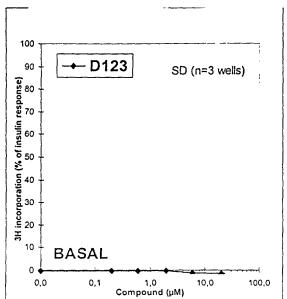


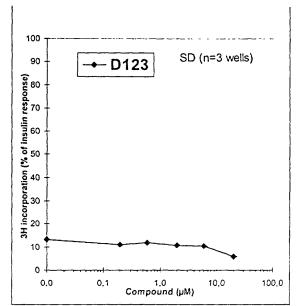
FIGURE 7102

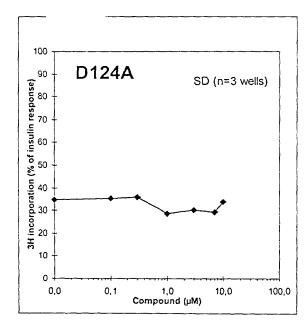
FIGURE 71P2

# FIGURE 71Q2



## FIGURE 71R2





Short the Art will have been the formal than the first

Harle Goods their three Stone Starte

FIGURE 71S2



### FIGURE 71U2

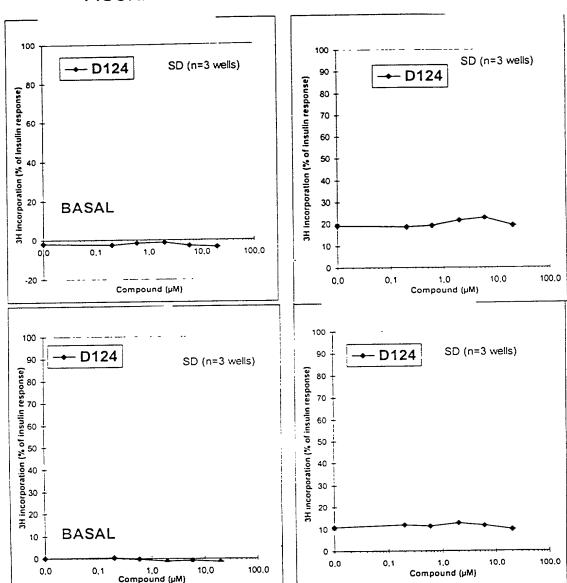
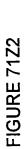
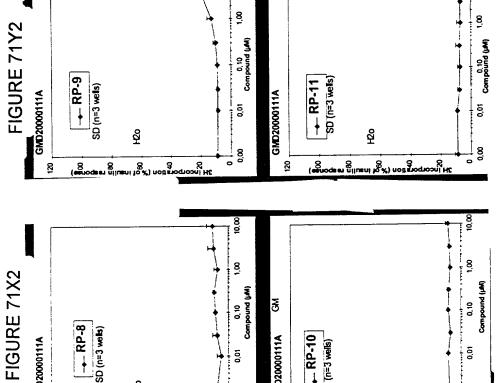


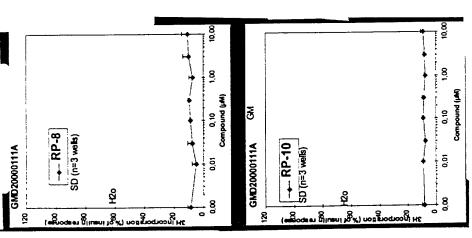
FIGURE 71V2

FIGURE 71W2



**FIGURE 71A3** 

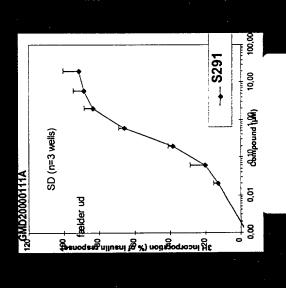




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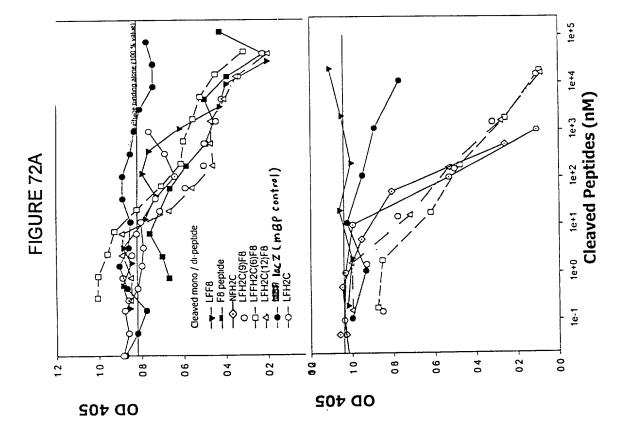
Linker 9 =

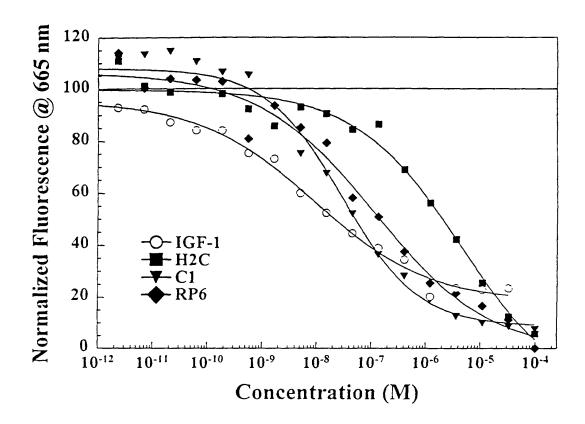
HIR binding =  $1.2*10^{-6}$ 



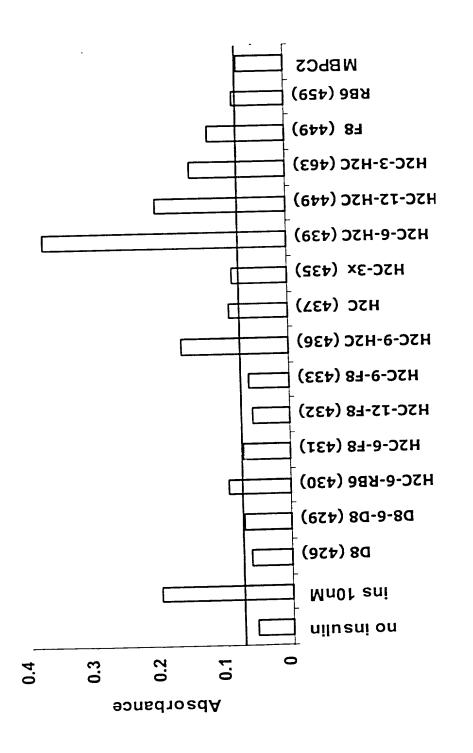
FFC:

FIGURE 71B3









# COMBINED DECLARATION AND POWER OF ATTORNEY FOR ORIGINAL, DESIGN, NATIONAL STAGE OF PCT, SUPPLEMENTAL DIVISIONAL, CONTINUATION OR CONTINUATION-IN-PART APPLICATION

As a below name inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

INSULIN AND	GF-1 RECEPTOR AGONIST	S AND ANTAGONIS	STS			
the specification	of which					
a. [X]	is attached hereto					
b. [ ]	was filed on as app	olication Serial No . (if applicable).	and was ar	mended on		
PCT FILED APPLICATION ENTERING NATIONAL STAGE						
c. [ ]	was described and claimed in and as amended on	International Applica (if any).	tion No.	filed on		
I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.						
I acknowledge the of Federal Regul	ne duty to disclose information ations, § 1.56.	which is material to the	ne patentability as defi	ned in Title 37, Code		
I hereby specify are to be directed	the following as the correspon	dence address to which	all communications	about this application		
SEND (	CORRESPONDENCE TO:	MORGAN & FINN 345 Park Avenue New York, N.Y. 10				
DIREC	T TELEPHONE CALLS TO:	(212) 758-4800				
[ ] I hereby claim foreign priority benefits under Title 35, United States Code § 119(a)-(d) or under § 365(b) of any foreign application(s) for patent or inventor's certificate or under § 365(a) of any PCT international application(s) designating at least one country other than the U.S. listed below and also have identified below such foreign application(s) for patent or inventor's certificate or such PCT international application(s) filed by me on the same subject matter having a filing date within twelve (12) months before that of the application on which priority is claimed:						
[ ] The this declaration.	e attached 35 U.S.C. § 119 clai	m for priority for the a	application(s) listed be	low forms a part of		
Country/PCT	Application Number	Date of filing (day, month, yr)	Date of Issue (day, month, yr)	Priority <u>Claimed</u>		
				[]YES []NO		

[ ] I hereby claim the benefit under 35 U.S.C. § 119(e) of any U.S. provisional application(s) listed below.

Provisional Application No.

Date of Filing (day, month, yr)

# ADDITIONAL STATEMENTS FOR DIVISIONAL, CONTINUATION OR CONTINUATION-IN-PART OR PCT INTERNATIONAL APPLICATION(S) (DESIGNATING THE U.S.)

I hereby claim the benefit under Title 35, United States Code § 120 of any United States application(s) or under § 365(c) of any PCT international application(s) designating the U.S. listed below.

U.S. Serial No. 09/146,127 September 2, 1998 Pending
US/PCT Application Serial No. Filing Date Status (patented, pending, abandoned)/
U.S. application no. assigned (For PCT)

[X] In this continuation-in-part application, insofar as the subject matter of any of the claims of this application is not disclosed in the above listed prior United States or PCT international application(s) in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or Imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

I hereby appoint the following attorneys and/or agents with full power of substitution and revocation, to prosecute this application, to receive the patent, and to transact all business in the Patent and Trademark Office connected therewith: John A. Diaz (Reg. No. 19,550), John C. Vassil (Reg. No. 19,098), Alfred P. Ewert (Reg. No. 19,887), David H. Pfeffer (Reg. No. 19,825), Harry C. Marcus (Reg. No. 22,390), Robert E. Paulson (Reg. No. 21,046), Stephen R. Smith (Reg. No. 22,615), Kurt E. Richter (Reg. No. 24,052), J. Robert Dailey (Reg. No. 27,434), Eugene Moroz (Reg. No. 25,237), John F. Sweeney (Reg. No. 27,471), Arnold I. Rady (Reg. No. 26,601), Christopher A. Hughes (Reg. No. 26,914), William S. Feiler (Reg. No. 26,728), Joseph A. Calvaruso (Reg. No. 28,287), James W. Gould (Reg. No. 28,859), Richard C. Komson (Reg. No. 27,913), Israel Blum (Reg. No. 26,710), Bartholomew Verdirame (Reg. No. 28,483), Maria C.H. Lin (reg. No. 29,323), Joseph A. DeGirolamo (Reg. No. 28,595), Michael P. Dougherty (Reg. No. 32,730), Seth J. Atlas (Reg. No. 32,454), Andrew M. Riddles (Reg. No. 31,657), Bruce D. DeRenzi (Reg. No. 33,676), Michael M. Murray (Reg. No. 32,537), Mark J. Abate (Reg. No. 32,527), Alfred L. Haffner, Jr. (Reg. No. 18,919), Harold Haidt (Reg. No. 17,509), John T. Gallagher (Reg. No. 35,516), Steven F. Meyer (Reg. No. 35,613), Kenneth H. Sonnenfeld (Reg. No. 33,285), Tony V. Pezzano (Reg. No. 38,271), Andrea L. Wayda (Reg. No. 43,979) and Walter G. Hanchuk Reg. No. (35,179) of Morgan & Finnegan, L.L.P. whose address is: 345 Park Avenue, New York, New York, 10154; and Michael S. Marcus (Reg. No. 31,727) and John E. Hoel (Reg. No. 26,279) of Morgan & Finnegan, L.L.P., whose address is 1775 Eye Street, Suite 400, Washington, D.C. 20006.

[ ]	I hereby authorize the U.S. attorneys and/or agents named hereinabove to accept and follow instructions from			
	as to any action to be taken in the U.S. Patent and Trademark Office			
	regarding this application without direct communication between the U.S. attorneys and/or agents and			
	me. In the event of a change in the person(s) from whom instructions may be taken I will so notify the			
	U.S. attorneys and/or agents hereinabove.			

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Full name of thirteenth joint inventor	Ku-chuan Hsiao	
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<del></del>	date	
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Citizenship		
	1 7 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	
Post Office Address 5 Wendover Roa	nd, Edison, New Jersey 08820, USA	<del></del>

- [ ] ATTACHED IS/ARE ADDED PAGE(S) TO COMBINED DECLARATION AND POWER OF ATTORNEY FORM FOR SIGNATURE BY FOURTH AND SUBSEQUENT INVENTORS
- * Before signing this declaration, each person signing must:
  - 1. Review the declaration and verify the correctness of all information therein; and
  - Review the specification and the claims, including any amendments made to the claims.

After the declaration is signed, the specification and claims are not to be altered.

To the inventor(s):

The following are cited in or pertinent to the declaration attached to the accompanying application:

#### Title 37, Code of Federal Regulation, § 1.56

Duty to disclose information material to patentability.

- A patent by its very nature is affect with a public interest. The public interest is best served, and the most effective patent examination occurs when, at the time an application is being examined, the Office is aware of and evaluates the teachings of all information material to patentability. Each individual associated with the filing and prosecution of a patent application has a duty of candor and good faith in dealing with the Office, which includes a duty to disclose to the Office all information known to that individual to be material to patentability as defined in this section. The duty to disclose information exists with respect to each pending claim until the claim is canceled or withdrawn from consideration, or the application becomes abandoned. Information material to the patentability of a claim that is canceled or withdrawn from consideration need not be submitted if the information is not material to the patentability of any claim remaining under consideration in the application. There is no duty to submit information which is not material to the patentability of any existing claim. The duty to disclose all information known to be material to patentability is deemed to be satisfied if all information known to be material to patentability of any claim issued in patent was cited by the Office or submitted to the Office in the manner prescribed by §§1.97(b)-(d) and 1.98. However, no patent will be granted on an application in connection with which fraud on the Office was practiced or attempted or the duty of disclosure was violated through bad faith or intentional misconduct. The Office encourages applicants to carefully examine:
- (1) prior art cited in search reports of a foreign patent office in a counterpart application, and



the closest information over which individuals associated with the filing or prosecution of a patent application believe any pending claim patentably defines, to make sure that any material information contained therein is disclosed to the Office.

#### Title 35, U.S. Code § 101

(2)

#### Inventions patentable

Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

#### Title 35 U.S. Code § 102

Conditions for patentability; novelty and loss of right to patent

A person shall be entitled to a patent unless -

- (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for patent,
- (b) the invention was patented or described in a printed publication in this or foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States, or
  - (c) he has abandoned the invention, or
- (d) the invention was first patented or caused to be patented, or was the subject of an inventor's certificate, by the applicant or his legal representatives or assigns in a foreign country prior to the date of the application for patent in this country on an application for patent or inventor's certificate field more than twelve months before the filing of the application in the United States, or
- (e) the invention was described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent, or on an international application by another who has fulfilled the requirements of paragraphs (1), (2), and (4) of section 371(c) of this title before the invention thereof by the applicant for patent, or
  - (f) he did not himself invent the subject matter sought to be patented, or
- (g) before the applicant's invention thereof the invention was made in this country by another had not abandoned, suppressed, or concealed it. In determining priority of invention there shall be considered not only the respective dates of conception and reduction to practice of the invention, but also the reasonable diligence of one who was first to conceive and last to reduce to practice, from a time prior to conception by the other ...

#### Title 35, U.S. Code § 103

Conditions for patentability; non-obvious subject matter

A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said matter pertains. Patentability shall not be negatived by the manner in which the invention was made.



Subject matter developed by another person, which qualifies as prior art only under subsection (f) or (g) of section 102 of this title, shall not preclude patentability under this section where the subject matter and the claimed invention were, at the time the invention was made, owned by the same person or subject to an obligation of assignment to the same person.

#### Title 35, U.S. Code § 112 (in part)

#### Specification

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise and exact terms also enable any person skilled in the art to which it pertains, or with which it is mostly nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention.

#### Title 35, U.S. Code § 119

Benefit of earlier filing date in foreign country; right of priority

An application for patent for an invention filed in this country by any person who has, or whose legal representatives or assigns have, previously regularly filed an application for a patent for the same invention in a foreign country which affords similar privileges in the case of applications filed in the United States or to citizens of the United States, shall have the same effect as the same application would have if filed in this country on the date on which the application for patent for the same invention was first filed in such foreign country, if the application in this country is filed within twelve months from the earliest date on which such foreign application was filed; but no patent shall be granted on any application for patent for an invention which had been patented or described in a printed publication in any country more than one year before the date of he actual filing of the application in this country, or which had been in public use or on sale in this country more than one year prior to such filing.

#### Title 35, U.S. Code § 120

Benefit or earlier filing date in the United States

An application for patent for an invention disclosed in the manner provided by the first paragraph of section 112 of this title in an application previously filed in the United States, or as provided by section 363 of this title, which is filed by an inventor or inventors named in the previously filed application shall have the same effect, as to such invention, as though filed on the date of the prior application, if filed before the patenting or abandonment of or termination of proceedings on the first application or an application similarly entitled to the benefit of the filing date of the first application and if it contains or is amended to contain a specific reference to the earlier filed application.

Please read carefully before signing the Declaration attached to the accompanying Application.

If you have any questions, please contact Morgan & Finnegan, L.L.P.

FORM:COMB-DEC.NY Rev. 5/21/98